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THE UNIVERSITY OF ALBERTA

I. NEW SYNTHESES OF 2'-DEOXYADENOSINES FROM ADENOSINE.

II. ACID-CATALYZED HYDROLYSIS AND CD SPECTRAL STUDIES ON 2'- AND 3'-O-SULFONYL AND CARBOXYLIC ESTERS OF ADENOSINE

BY

© PETER SPORNS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA
SPRING, 1977

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled

"I. NEW SYNTHESES OF 2'-DEOXYADENOSINES FROM ADENOSINE.
II. ACID-CATALYZED HYDROLYSIS AND CD SPECTRAL STUDIES ON 2'- AND 3'-O-SULFONYL AND CARBOXYLIC ESTERS OF ADENOSINE"

submitted by PETER SPORNS in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Morris J. Robins
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External Examiner

Date. December 10, 1976

ABSTRACT

A procedure to synthesize 2'-deoxyadenosine (and 3'-deoxyadenosine) directly from adenosine was developed. This involved preparation of 9-(2,3-O-methoxyethylidene-β-D-ribofuranosyl)adenine followed by selective acetylation of the 5'-hydroxyl. Ortho ester hydrolysis of the resulting compound in acid afforded both 9-(3,5 di-O-acetyl-β-D-ribofuranosyl)adenine and 9-(2,5 di-O-acetyl-β-D-ribofuranosyl)adenine, with the former predominating. This mixture was chlorinated at the respective free hydroxyl positions by treatment with thionyl chloride in hot pyridine. Subsequent reduction with tri-n-butyltin hydride, followed by deblocking, gave 2'-deoxyadenosine and a lesser amount of 3'-deoxyadenosine which were readily separated on a Dekker column [Dowex 1-X2 (OH^-)]. Although the overall yield was somewhat low, this method did give considerably better yields than existing techniques. A similar scheme with modifications in the blocking-deblocking procedures was used to prepare 2'-deoxy-3'-O-methyladenosine from 3'-O-methyladenosine.

A series of 2'- and 2'-O-sulfonyl and carboxylic esters of adenosine were synthesized to study their physical properties. The esters studied included: methanesulfonyl, benzylsulfonyl, p-toluenesulfonyl, p-nitrobenzenesulfonyl, p-aminobenzenesulfonyl, trifluoromethanesulfonyl, p-methoxyphenylacetyl and p-methoxybenzoyl. In addition some properties of the 2'- and 3'-O-methyl and benzyl ethers of adenosine were investigated. Measurement of the ultra violet and circular dichroism spectra of these compounds was useful in determining the extent of base, sugar-substituent overlap. It was found that only 2' aromatic sugar substituents have observable electronic interaction with the base in the series of compounds studied. Base, sugar-substituent overlap was also found to be dependent on the steric constraints of the sugar substituent. Acid-catalyzed hydrolysis of the base-glycosyl bond of these adenosine derivatives showed a remarkable enhancement of stability when electronegative sugar substituents were present. This was especially true when the electronegative substituent was at the 2' position. Steric effects were found to have some effect on stabilization of the base-glycosyl bond in acid but base, sugar-substituent overlap was shown to be insignificant in this stabilization. The nuclear magnetic resonance and mass spectra of these compounds also displayed interesting trends which are discussed.

ACKNOWLEDGEMENTS

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Last but certainly not least my wife Susan and son Ryan for their love.

TABLE OF CONTENTS

PAGE

INTRODUCTION

A BRIEF HISTORICAL INTRODUCTION TO NUCLEOSIDES 1
INTRODUCTION TO THE SYNTHESIS OF 2-DEOXY NUCLEOSIDES 4
INTRODUCTION TO CIRCULAR DICHROISM OF NUCLEOSIDES 11
INTRODUCTION TO ACID-CATALYZED HYDROLYSIS OF THE BASE-GLYCOSYL BOND OF NUCLEOSIDES........... 23

DISCUSSION AND RESULTS

SYNTHESIS OF 2'-DEOXY ADENOSINE COMPOUNDS....... 30
SYNTHESIS OF 2' AND 3' SULFONYL AND CARBOXYLIC ESTERS OF ADENOSINE.................. 40
ULTRAVIOLET AND CIRCULAR DICHROISM SPECTRA...... 46
ACID-CATALYZED HYDROLYSIS OF THE BASE-GLYCOSYL BOND OF SOME ADENOSINE DERIVATIVES.............. 60
MASS SPECTRA.................................... 69
NUCLEAR MAGNETIC RESONANCE SPECTRA.............. 76

EXPERIMENTAL

GENERAL PROCEDURES.............................. 81
HYDROLYSIS...................................... 84
SYNTHESIS....................................... 90
REFERENCES... 145
APPENDIX... 159

LIST OF TABLES

TABLE		PAGE
1.	Acid Hydrolysis Data on 2' and 3'-O Substituted Adenosines	61
2.	^{1}H Nmr Data for Sugar Substituted Adenosine Derivatives	79
3.	Extinction Coefficients at 254 nm of Sugar Substituted Adenosine Derivatives	89

LIST OF FIGURES

FIGURE	DESCRIPTION	PAGE
1.	Commonly Occurring Nucleosides	2
2.	Selected Syntheses of 2'-Deoxy Nucleosides	5
3.	Ikehara's Synthesis of 2'-Deoxyadenosine from Adenosine	9
4.	Vectorial Representation of Optical Rotatory Dispersion and Circular Dichroism Effects	14
5.	Typical Optical Rotatory Dispersion and Circular Dichroism Spectra	15
6.	Ultraviolet and Circular Dichroism Interaction Possible with Two Chromophores	19
7.	Tortional Angle of Nucleosides	21
8.	Acid-Catalyzed Hydrolysis Mechanisms for the Base-Glycosyl Bond	24
9.	Synthesis of 9-(3-O-Methyl-5-O-pivalyl-β-D-ribofuranosyl)adenine (22)	31
10.	Synthesis of 9-(2-Deoxy-3-O-methyl-β-D-ribofuranosyl)adenine (24)	35
11	Synthesis of 2'-Deoxyadenosine (10)and 3'-Deoxyadenosine (31)	37
12.	Synthesis of Sulfonyl Esters of 2'-O-Methyladenosine (32) and 3'-O-Methyladenosine (17)	41
13.	9-(2-O-Benzyl-β-D-ribofuranosyl)adenine (47) and 9-(3-O-Benzyl-β-D-ribofuranosyl)-adenine (46)	43
14.	Synthesis of Carboxylic Esters of 2'-O-Methyladenosine (32) and 3'-O-Methyladenosine (17)	45
15.	Circular Dichroism Spectra of 2'-O-Methyladenosine (32), 3'-O-Methyladenosine (17), 9-(2'O-Benzyl-β-D-ribofuranosyl)adenine (47) and 9-(3-O-Benzyl-β-D-ribofuranosyl)adenine (46)	49

FIGURE	DESCRIPTION	PAGE
16	Circular Dichroism Spectra of 9-(3-O-Methyl-2-O-trifluoromethanesulfonyl-β-D-ribofuranosyl)adenine (44), 9-(3-O-Methyl-2-O-methanesulfonyl-β-D-ribofuranosyl)adenine (34), 9(2-O-Methyl-3-O-methanesulfonyl-β-D-ribofuranosyl)adenine (35) and 9(2-O-Methyl-3-O-trifluoromethanesulfonyl-β-D-ribofuranosyl)adenine (45)	51
17.	Circular Dichroism Spectra of 9(3-O-Methyl-2-O-p-toluenesulfonyl-β-D-ribofuranosly)adenine (38), 9-(3-O-Methyl-2-O-benzylsulfonyl-β-D-ribofuranosyl)adenine (36), 9(2-O-Methyl-3-O-p-toluenesulfonyl-β-D-ribofuranosyl)adenine (39) and 9(2-O-Methyl-3-O-benzylsulfonyl-β-D-ribofuranosyl)adenine (37)	52
18.	Circular Dichroism Spectra of 9(3-O-Methyl-2-O-p-aminobenzenesulfonyl-β-D-ribofuranosyl)adenine (42), 9(3-O-Methyl-2-O-p-nitrobenzenesulfonyl-β-D-ribofuranosyl)adenine (40), 9(2-O-Methyl-3-O-p-aminobenzenesulfonyl-β-D-ribofuranosyl)adenine (43 and 9(2-O-Methyl-3-O-p-nitrobenzenesulfonyl-β-D-ribofuranosyl)adenine (41)	55
19.	Circular Dichroism Spectra of 9(3-O-Methyl-2-O-p-toluenesulfonyl-β-D-ribofuranosyl)adenine (38) and 9(3-O-Methyl-2-O-p-aminobenzenesulfonyl-β-D-ribofuranosyl)adenine (42) at pH = 6.2 and pH = 1.1.	56
20.	Circular Dichroism Spectra of 9(3-O-p-Methoxybenzoyl-2-O-methyl-β-D-ribofuranosyl)adenine (51), 9(2-O-p-Methoxybenzoyl-3-O-methyl-β-D-ribofuranosyl)adenine (50), 9(2-O-p-Methoxyphenylacetyl-3-O-methyl-β-D-ribofuranosyl)adenine (48) and 9(3-O-p-Methoxyphenylacetyl-2-O-methyl-β-D-ribofuranosyl)adenine (49)	58
21.	Acid-Catalyzed Hydrolysis of 9(3-O-Methyl-2-O-trifluoromethanesulfonyl-β-D-ribofuranosyl)adenine (44)	66

FIGURE	DESCRIPTION	PAGE
22.	Acid-Catalyzed Hydrolysis of 9(2-O-Methyl-3-O-trifluoromethanesulfonyl-β-D-ribofuranosyl)adenine (45)	67
23.	Proposed Formation of Mass Spectral Fragments c, x and h	70
24.	Proposed Formation of Mass Spectral Fragments d, e, f and i	73

INTRODUCTION

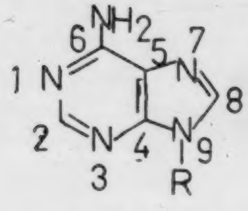
A BRIEF HISTORICAL INTRODUCTION TO NUCLEOSIDES

In 1871 F. Miescher [1] isolated a material which he called nuclein, from pus cells (obtained from discarded surgical bandages). Altman [2] first introduced the term "nucleic acid" in 1889 when he successfully isolated nucleic acids from a number of materials. In 1891, Kossel [3] reported the first results of hydrolyses of nucleic acids. This led to the identification and synthesis of the five major naturally occurring heterocyclic bases by Kossel, Fischer, Traube and Steudel at the beginning of the twentieth century.[4] These are, in the case of ribonucleic acid (RNA), the purines, adenine (1) and guanine (2) and the pyrimidines, cytosine (3) and uracil (5). In deoxyribonucleic acid (DNA) the same major bases occur with the exception of uracil which is replaced by thymine (4).

The term nucleoside was introduced by Levene and Jacobs [5] to designate the purine-carbohydrate derivatives isolated from alkaline hydrolysis of yeast ribonucleic acid. This term is now commonly used to describe a heterocyclic base attached to the C-1 position of a sugar.

Although in his earlier work Kossel suggested that acidic hydrolysis of RNA liberated a carbohydrate

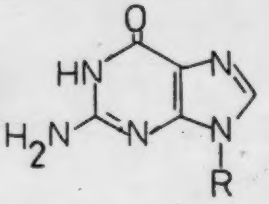
Figure 1


6
1
2
3
4
5
7
8
9
NH2
N
R

1 R=H

6 R=R′

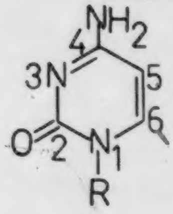
10 R=R″



2 R=H

7 R=R′

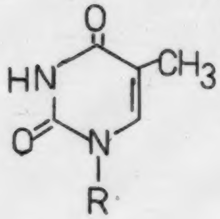
11 R=R″


NH2
4
3N
5
6
O
2
N1
R

3 R=H

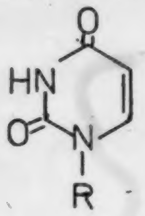
8 R=R′

12 R=R″



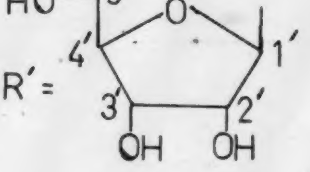
4 R=H

13 R=R″

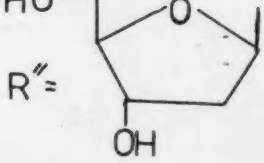


5 R=H

9 R=R′


HO
5′
O
4′
1′
R′ =
3′
2′
OH
OH

D-RIBOSE



2-DEOXY-D-RIBOSE

derivative, it was not until many years later that Levene and Jacobs[6] correctly characterized it as D-ribose. Still later Levene and Mori identified the sugar present in DNA[7] as 2-deoxy-D-ribose. Levene and Tipson[8] subsequently identified the furanosyl structure of the ribose moiety using methylation studies.

The position of attachment of the heterocyclic base to the sugar was established by ultraviolet spectral comparisons[9-11] and further confirmed by Todd and coworkers[12], by comparison of products resulting from periodate oxidation of natural and synthetic compounds. Later Todd and coworkers[13] also identified the configuration of base attachment to the sugar as beta (β).

Thus the commonly occurring nucleosides found in RNA are 9-(β-D-ribofuranosyl)adenine (adenosine (6)), 9-(β-D-ribofuranosyl)guanine (guanosine (7)), 1-(β-D-ribofuranosyl)uracil (uridine (9)) and 1-(β-D-ribofuranosyl)cytosine (cytidine (8)). In DNA they are 9-(2-deoxy-β-D-ribofuranosyl)adenine (2'-deoxyadenosine (10)), 9-(2-deoxy-β-D-ribofuranosyl)guanine (2'-deoxyguanosine (11)), 1-(2-deoxy-β-D-ribofuranosyl)cytosine (2'-deoxycytidine (12)) and 1-(2-deoxy-β-D-ribofuranosyl)thymine (thymidine (13)).

The foregoing material has been the subject of several extensive reviews.[14-20]

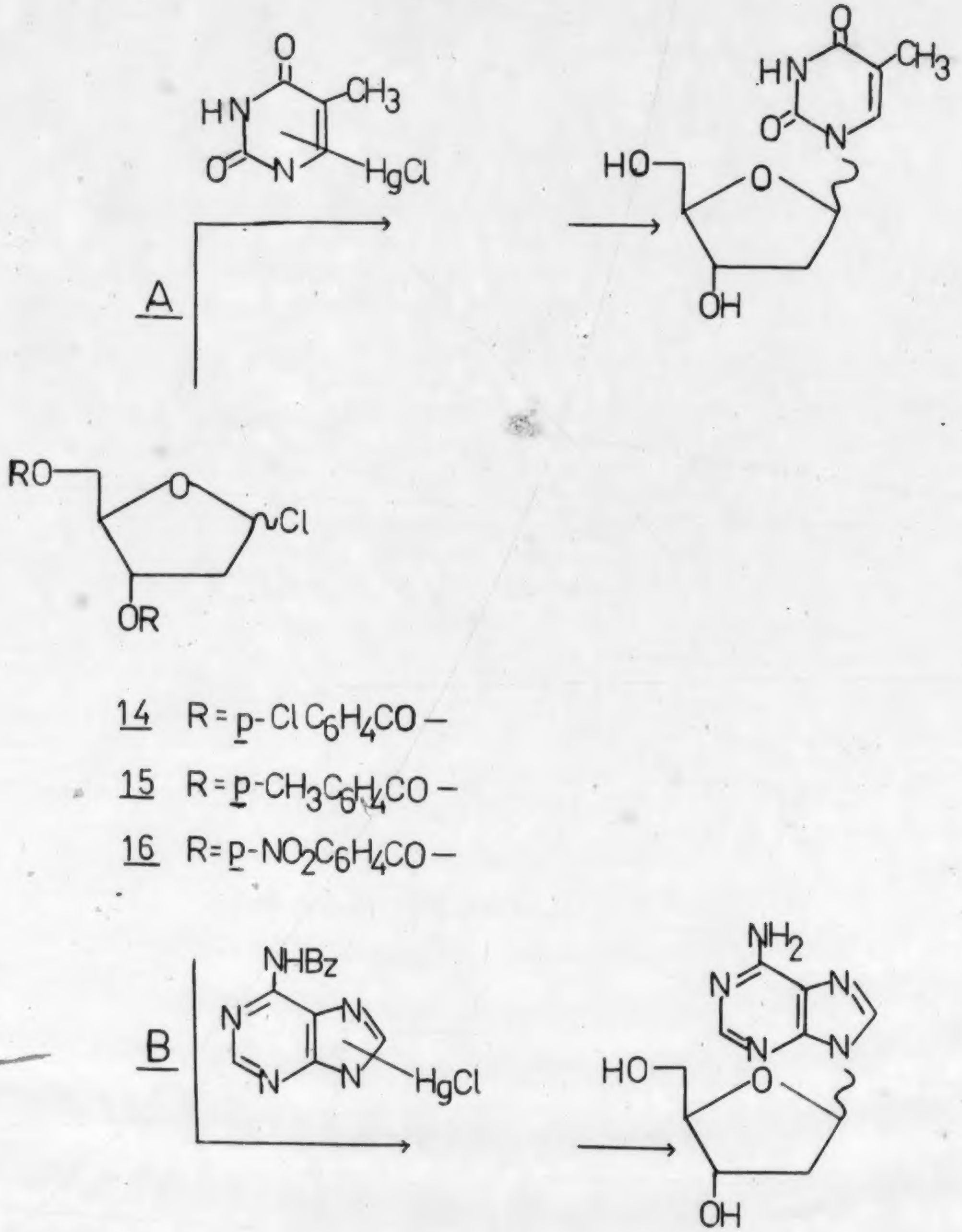
INTRODUCTION TO THE SYNTHESIS OF 2'-DEOXY NUCLEOSIDES

Initial attempts to carry out base-sugar coupling reactions to prepare 2'-deoxynucleosides met with failure due to the extreme lability of the poly-*O*-acyl-2-deoxyglycosyl halides.

The first workers to successfully prepare 2'-deoxynucleosides in a coupling reaction used 2-deoxy-D-ribofuranosyl chloride stabilized by protection with *para* substituted benzoyl blocking groups.[21,22] They used the mercury salt coupling procedure [23,24] which had been developed for the synthesis of ribonucleosides. Hoffer and coworkers [21,25] used *p*-chloro-(**14**) or *p*-methylbenzoyl-2-deoxy-D-ribosyl chloride (**15**) and obtained good yields of α and β anomeric deoxynucleosides of thymine, 5-fluorouracil and cytosine (using *N*-acetyl protected cytosine). (See method A, Figure 2). Ness and Fletcher [22] successfully prepared α and β anomers of 2'-deoxyadenosine in low yields, after deblocking, by coupling the chloromercuri derivative of 6-benzamidopurine with p-nitrobenzoyl-2-deoxy-D-ribosyl chloride (**16**). (See method B, Figure 2).

These first studies were followed by a series of publications by other workers who used similar procedures.[26-31] Later authors modified the above procedure by using trimethylsilyl derivatives of bases rather than

Figure 2


A
HgCl
CH3
HN
O
N
RO
Cl
OR
HO
OH
B
NHBz
N
HgCl
NH2

14 R = $\underline{p}$-ClC_6H_4CO–

15 R = $\underline{p}$-$CH_3C_6H_4CO$–

16 R = $\underline{p}$-$NO_2C_6H_4CO$–

mercury salts to prepare 2'-deoxynucleosides. This method was systematically studied by Nishimura [32,33] for ribonucleosides and was used widely in later publications for preparation of 2'-deoxy derivatives.[34-41] Other authors used bases with the cyclic amide carbonyls protected as lactim ether groups [42,43] as Hilbert and Johnson had first developed in their classical coupling technique.[44] Still others fused totally acetylated 2'-deoxysugars, rather than glycosyl halides, with suitably protected bases.[45-48] This procedure was developed by a number of Japanese investigators [49] for ribonucleosides and was first applied to the synthesis of 2'-deoxynucleosides by Robins and Robins.[46]

All of the above techniques suffer from the fact that both α and β anomers are formed and must be separated. In the case of 2-O-acyl substituted sugars Baker [50] noted that condensation reactions of glycosyl halides with metal salts of purine or pyrimidine bases gave nucleosidic products having only the trans C-1', C-2' configuration. Thus one product results from these condensations. This has been explained [50,51] in terms of neighbouring group participation by the 2-O-acyl group of the glycosyl halide.

Recently Bardos and coworkers [52] have attempted to control the anomeric ratio using the pure α-anomer of 14. Reaction conditions allowing rapid removal of the trimethylsilyl chloride formed in the reaction (using trimethylsilylated base) led to the β-anomer as the only isolatable nucleosidic product, whereas, continued presence of the trimethylsilyl chloride favoured the formation of the α-anomer.

Also Ryan, Acton and Goodman [53] have successfully synthesized 2'-alkylthiopurine nucleosides using alkyl 1-thio-α-D-arabinofuranosinde as the sugar precursor. This reaction was thought to proceed through an episulfonium ion which forms between C-1 and C-2. Trip [54] has recently modified the coupling procedure and reduced the alkylthio group to successfully prepare some 2'-deoxy nucleosides. However, he found that the final step, reductive removal of the alkylthio group, severely limited the yield using this procedure.

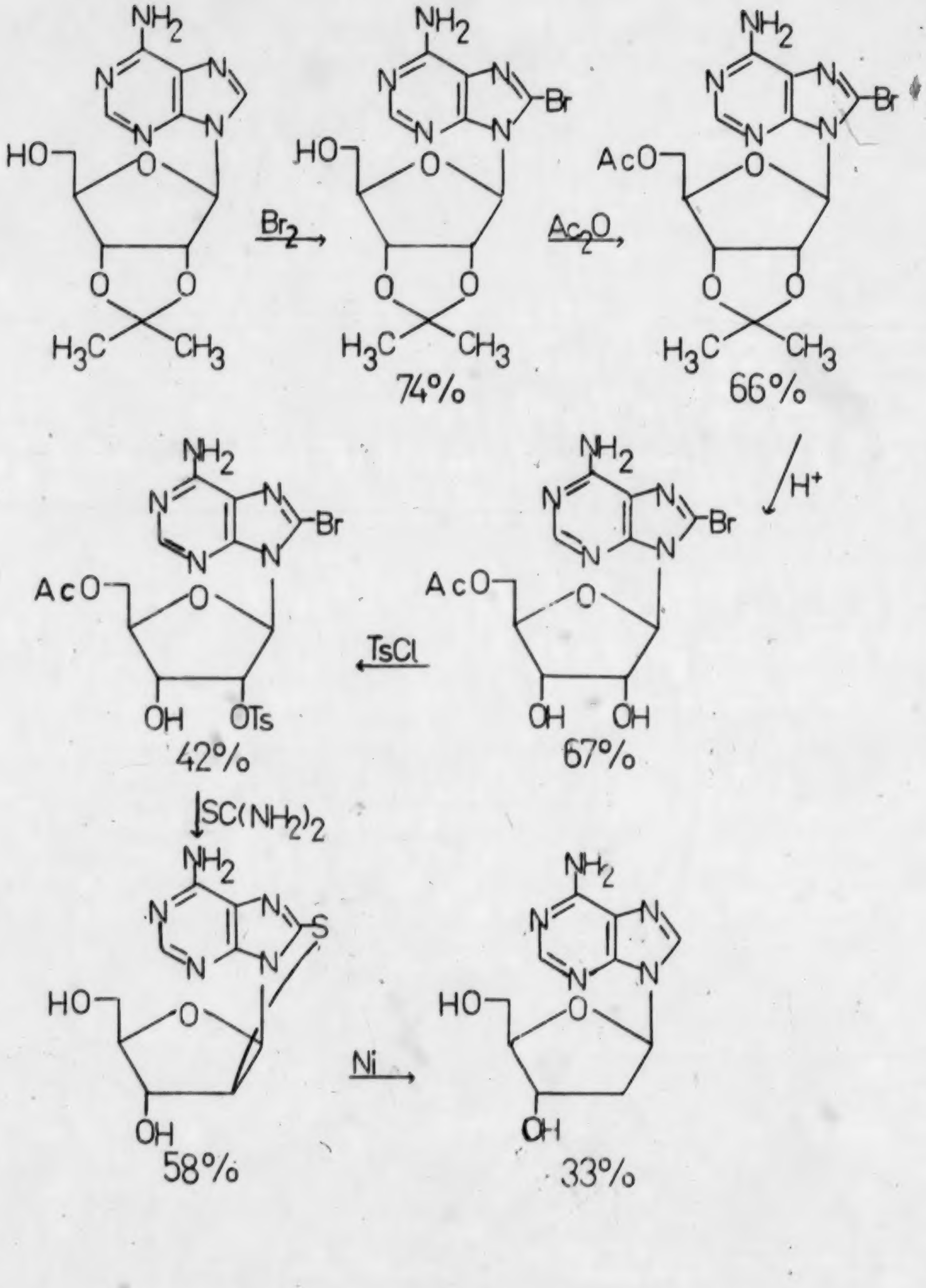
Rather than using a coupling technique, many workers have attempted to modify preformed nucleosides to form 2'-deoxy nucleosides. Todd and coworkers [55] reported the first synthesis of a pyrimidine 2'-deoxynucleoside. These workers found that treatment of 5'-O-acetyl-2'-O-p-toluenesulfonyluridine with sodium iodide in acetonylacetone gave the 2'-iodo derivative which after hydrogenation and deacylation gave 2'-deoxyuridine. They showed that the reaction went through an O^2, 2'-anhydropyrimidine derivative. There followed several papers using anhydropyrimidine derivatives to synthesize pyrimidine 2'-deoxynucleosides.[56-58]

Holý [59-61] has employed O^2, 2'-anhydropyrimidine nucleosides, building up these compounds from sugar amino-oxazolines in a procedure originally developed by Sanchez and Orgel.[62] He then proceeded using known reactions to the 2'-chloro products which he reduced with tri-*n*-butyltin hydride.

Syntheses of purine 2'-deoxynucleosides are much more difficult and generally give very low yields. Ikehara and coworkers [63,64] synthesized 8-2'-anhydro-8-mercaptopurine derivatives *via* the 8-bromo compounds (see Figure 3). These could be desulfurized with Raney nickel to give the corresponding 2'-deoxynucleosides. This procedure was long and proceeded in poor overall yield (2'-deoxyadenosine from adenosine in ~2% overall yield). Again, the final reductive desulfurization proceeded in a low 33% yield.

Both Robins and coworkers [65,66] and Moffatt and coworkers [67] have prepared purine 2'-halonucleosides which can be reduced to 2'-deoxynucleosides, but the 2'-halo derivatives are only minor products (3'-halo derivatives predominate). In a long and extensive procedure by Goodman and coworkers [68-70] 2'-deoxyadenosine was synthesized from 3'-deoxy-3'-ethylthio-9-β-*D*-xylofuranosyladenine. Migration of the 3'-ethylthio residue to the 2'-position, *via* a 2', 3'-episulfonium ion,

Figure 3


Br$_2$
74%
Ac$_2$O
66%
H$^+$
TsCl
67%
42%
SC(NH$_2$)$_2$
58%
Ni
33%

yielded a 2'-ethylthio-3'-chloro derivative. Hydrolysis and subsequent desulfurization gave mixtures of 2'- and 3'-deoxyadenosine with the 2' isomer predominating. Goodman[70] again found that desulfurization in the final step drastically reduced the already small overall yields.

To summarize then, in the case of pyrimidine nucleosides, reaction through the $\underline{O}^2$,2'-anhydropyrimidine derivatives yields the corresponding 2'-deoxypyrimidines in good yields. There is as yet, however, no corresponding transformation (that proceeds in good yield) available in the purine nucleoside series.

INTRODUCTION TO CIRCULAR DICHROISM OF NUCLEOSIDES

To consider the phenomenological basis of circular dichroism (cd) it is necessary to first consider optical rotatory dispersion (ord). Specific rotation [α], especially at the wavelength of the sodium D line (589 nm), has long been used to identify and help establish the configuration of optically active compounds. Historically the equation used for specific rotation is:

$$[\alpha]_\lambda^t = \alpha \times 100/l \times c'$$

where α is the observed rotation in degrees, t is the temperature, λ is the wavelength at which the rotation is measured, l is the light path length through the solution in decimeters and c' is the concentration in grams per 100 ml of solution. A more recent expression which incorporates molecular weight is the molar rotation [ϕ]:

$$[\phi]_\lambda = [\alpha]_\lambda \times M/100$$

where M is the molecular weight of the solute.

It is remarkable that over the years specific rotation has been so useful, since it was limited to only a few wavelengths at most. Ord is simply the dependence of [ϕ] on the wavelength of light. Thus, just as [α] or [ϕ] are a measure of the specific rotation of the plane of polarized light at one wavelength, ord is a measure over a large range of wavelengths.

Monochromatic plane polarized light is a special type of polarization in which a left circularly polarized component (El) and a right circularly polarized component (Er) have opposite rotation senses but the same amplitude. If plane polarized light passes through an optically active medium El and Er pass throughat different velocities depending on the circular birefringence $(n_L - n_R)$ exhibited by the medium. This leads to a change of phase correlation and results in a rotation [α] of the plane of polarization.

If the optically active molecule has a chromophore near the optically active center, a new effect appears. Most chromophores are optically inactive themselves because of their π structure (usually planar). If the chromophore is close to an optically active center, the dissymmetry of the environment causes a difference in the absorption of El and Er depending on the circular dichroism $(k_l - k_r)$ exhibited by the compound. This causes a change in relative amplitude as well as velocity of the circularly polarized components and produces a wave which is elliptically polarized.

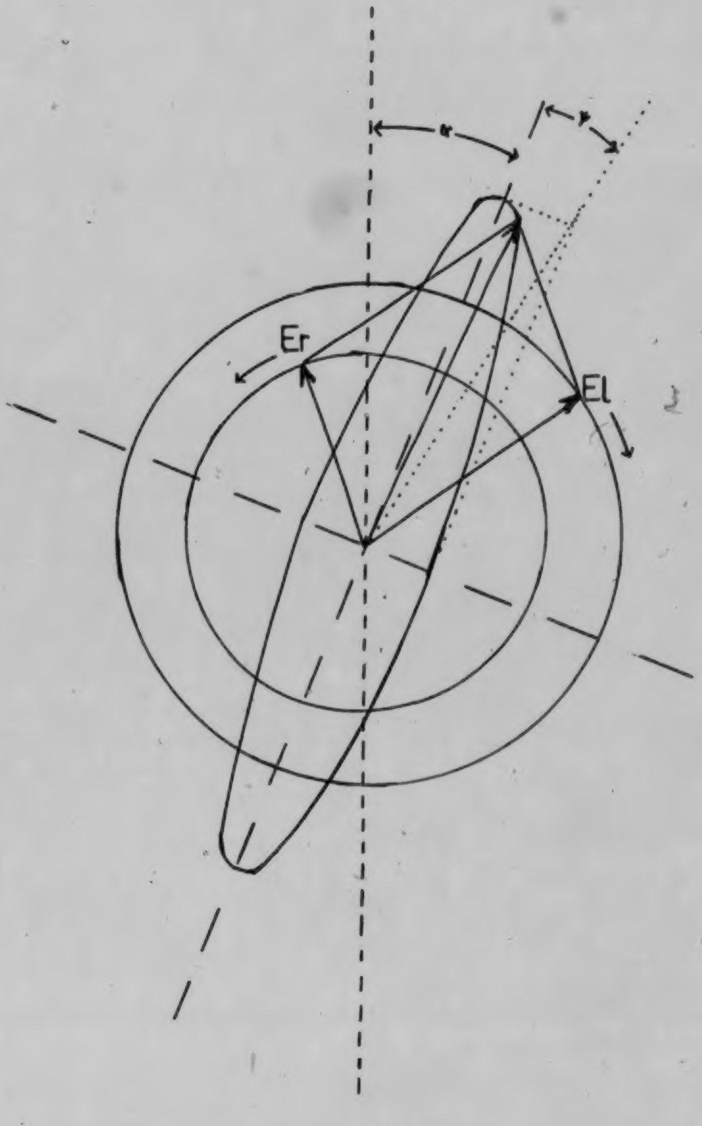
In the vicinity of a chromophore, a compound exhibits the combination of circular birefringence (ord) and circular dichroism (cd) and besides rotating the plane of polarization by angle α, the linear plane of polarized light becomes elliptical. The angle of ellipticity (ψ) is defined as the arc-tangent of the ratio of minor to major axis of the elliptical vibration. (See Figure 4).

In the region of the chromophore, the ord exhibits an "anamolous dispersion" which is now called the Cotton effect after its discoverer.[71,72] That is, in the case of a positive Cotton effect the ord has a positive optical rotation at longer wavelength values and a negative rotation at shorter wavelengths. The crossover point, in the ideal case of an electronic transition well separated from all others, is at the ultraviolet (uv) absorption maximum. A negative Cotton effect has the opposite configuration (i.e. negative rotation at longer wavelength and positive at shorter wavelength).

Corresponding to the positive and negative Cotton effect in ord is a positive and negative (respectively) cd. This is a measure of the difference in absorbance of El and Er and so can only occur in the region of a chromophore. Again in the ideal case, the maximum in the cd curve is at the same wavelength as the uv maximum. (See Figure 5.).

Cd is usually expressed as the molar coefficient of dichroic absorption ($\Delta\varepsilon$), where k_l and k_r (the absorption indices) can be replaced by the molar extinction coefficients ε_l and ε_r by the relationship:

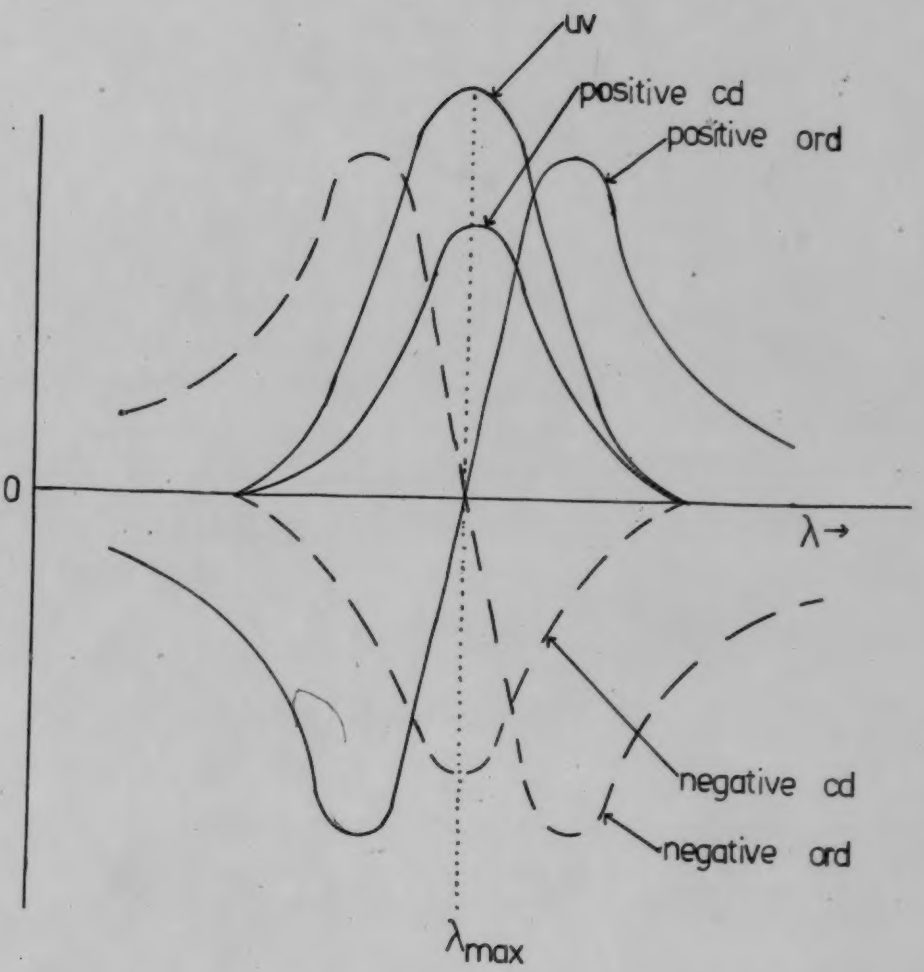
Figure 4



$\alpha = \frac{\pi}{\lambda}(n_l - n_r)$ $\psi = \frac{\pi}{\lambda}(k_l - k_r)$

$n_l(k_l)$ are refractive (absorption) indices for left circularly polarized light

Figure 5


uv
positive cd
positive ord
0
λ→
negative cd
negative ord
λ_{max}

$$k = \frac{2.303}{4\pi} \times \lambda c \varepsilon$$

where c is the concentration of the absorbing solute in moles per liter and λ is in centimeters. Another expression often used to describe cd is molar ellipticity $[\phi]$:

$$[\phi] = 3300\, \Delta\varepsilon$$

Ord was used more frequently in earlier literature because of the experimental difficulties involved in measuring cd. It was not until 1967 that commercial instruments sensitive enough to measure the cd of nucleosides were available. The cd of nucleosides are especially difficult to determine since they have very large extinction coefficients and low optical rotations, resulting in a small signal to noise ratio. With improvements in instrumentation cd has become increasingly popular due to its more simple spectral characteristics relative to ord. This is especially true for complex spectra (nucleosides) where there are many overlapping transitions and multiple Cotton effects. The above material is thoroughly reviewed in several sources.[19,73-75]

In cases involving interaction of two chromophores, the uv and cd spectra can become more complex. The type of effects noted can be divided into two main groups. The first type of effect is exciton coupling. This is a resonance effect between chromophores. Exciton coupling can be further divided into cases of strong coupling and weak coupling. In both cases the theory has been derived assuming identical chromophores and then applying the resulting equations to non-identical chromophores. Exciton coupling results in complete splitting of the uv band, with retention of overall intensity, into two separate bands in the case of strong exciton coupling. In weak exciton coupling (more applicable to polynucleotides) the uv band is broadened with retention of intensity. In each case the resulting cd is conservative. A negative and positive cd result, which when integrated and added together equal zero. The cd bands formed correspond to the split uv band.

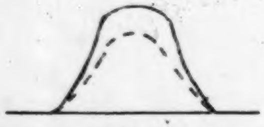
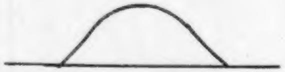
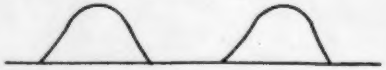
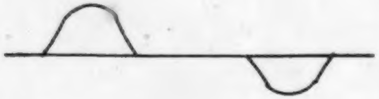
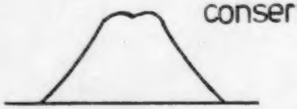
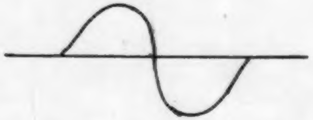
The second major case is a more generally observed effect when two chromophores interact. In this nonresonant case, the interaction between two chromophores is simply the Coulomb interaction between their electrons. Small changes occur in the excited states due to the polarization of the neighbouring chromophore by incident light. A polarized neighbouring chromophore creates a field, at the chromophore being considered, which adds to the incident electric field. Thus for this chromophore the polarizability of the environment is anisotropic since the neighbouring chromophore lies in one direction whereas the solvent molecules lie in the other direction. This anisotropic polarization modifies the absorbance of the primary chromophore, giving hypochromism (decrease in ε max) for $\pi \rightarrow \pi^*$ transitions (horizontal or parallel to the plane of the base) and hyperchromism (increase in ε max) for $n \rightarrow \pi^*$ transitions (vertical or perpendicular to the plane of the base).

The analogous effect occurs in the cd of stacked chromophores. If the polarizabilities differ for El and Er, there will be different hypochromism effects for the two polarizations giving rise to a nonconservative (non zero when integrated) cd. (See Figure 6).

These uv and cd effects (in the second major case) can occur for identical as well as for nonidentical neighbouring chromophores and are in no way related to resonance energy transfer. A more complete discussion of these effects appears in several reviews.[74-77]

Cd spectra are also influenced by other changes in the molecule. One example is a change in the chromophore due to either an actual chemical modification or ionization [78,79] which will often markedly affect the cd spectrum (and the uv spectrum). Another effect that must be considered is overlapping of cd curves due to more than one chromophore or multiple transitions in a single chromophore. In adenine, for example, the $\pi \rightarrow \pi^*$ transitions are thought to occur at ~260 nm, ~240 nm and ~220 nm.[80] In addition there are several possible $n \rightarrow \pi^*$

Figure 6

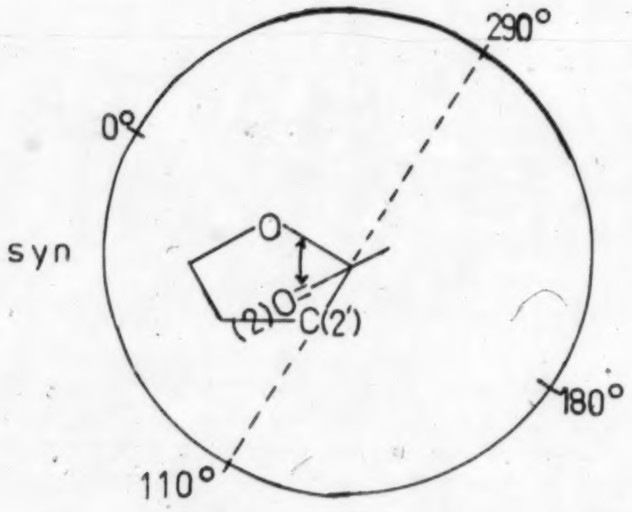
	Interaction effect on:		
Dimer Interaction	Absorption	cd	Energy transfer
Nonresonant interaction	Hypochromism 	Nonconservative cd 	no
Strong resonant interaction	Exciton splitting, Total intensity conserved 	Conservative cd 	yes
Weak resonant interaction	Exciton splitting, Intensity conserved 	Conservative cd 	yes

transitions [81] which could also contribute to the overall cd spectrum.

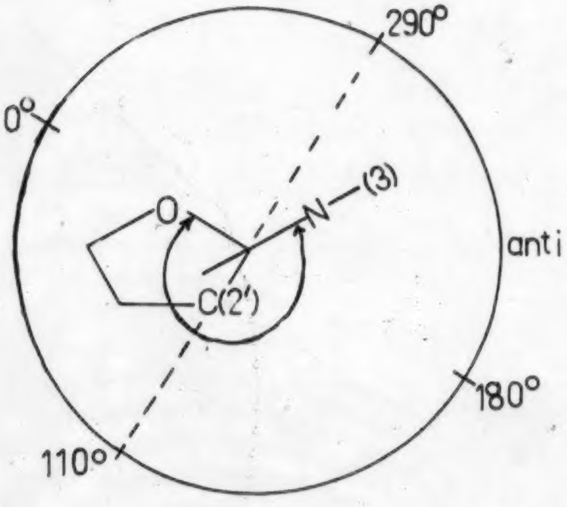
Another source of modification of the cd effect is variation in sugar conformation and especially variation in the torsional angle (χ_{CN}).[19] The torsional angle is defined as the angle between the plane of the base (specifically the N-3 of a purine or the 2 carbonyl of a pyrimidine base) and the plane of the C-1 to ring oxygen of the sugar. If this angle is between 290° to 110°, the base is said to be in the syn conformation and if the angle is between 110° to 290°, the base is said to be in the anti conformation.[82] (See Figure 7). Sundaralingam [83] defines the torsional angle χ_{CN} as anti when in the range 0° ± 90° and syn as in the range 180° ± 90°. Donahue and Trueblood [84] defined torsional angle (ϕ_{CN} in their case) with a positive rotation from 0° to 180° clockwise and a negative rotation from 0° to 180° counterclockwise. The effect of this angle on the cd curve magnitude and sign for adenine nucleosides has recently been studied by Bush.[85] It has also been shown that restricted rotation of the base (in arabinofuranosyl compounds for example) causes an enhancement of the cd effect.[86,87]

Besides the above effects causing changes in the cd, solvent,[88,89] concentration, temperature, and salt effects can also affect the cd spectrum. Thus detailed

Figure 7


290°
0°
syn
O
(2)O
C(2')
180°
110°

pyrimidine (syn)


290°
0°
O
N—(3)
anti
C(2')
180°
110°

purine (anti)

quantitative interpretation of a cd spectrum is often difficult if not impossible but a qualitative investigation of a cd spectrum can yield valuable structural information.

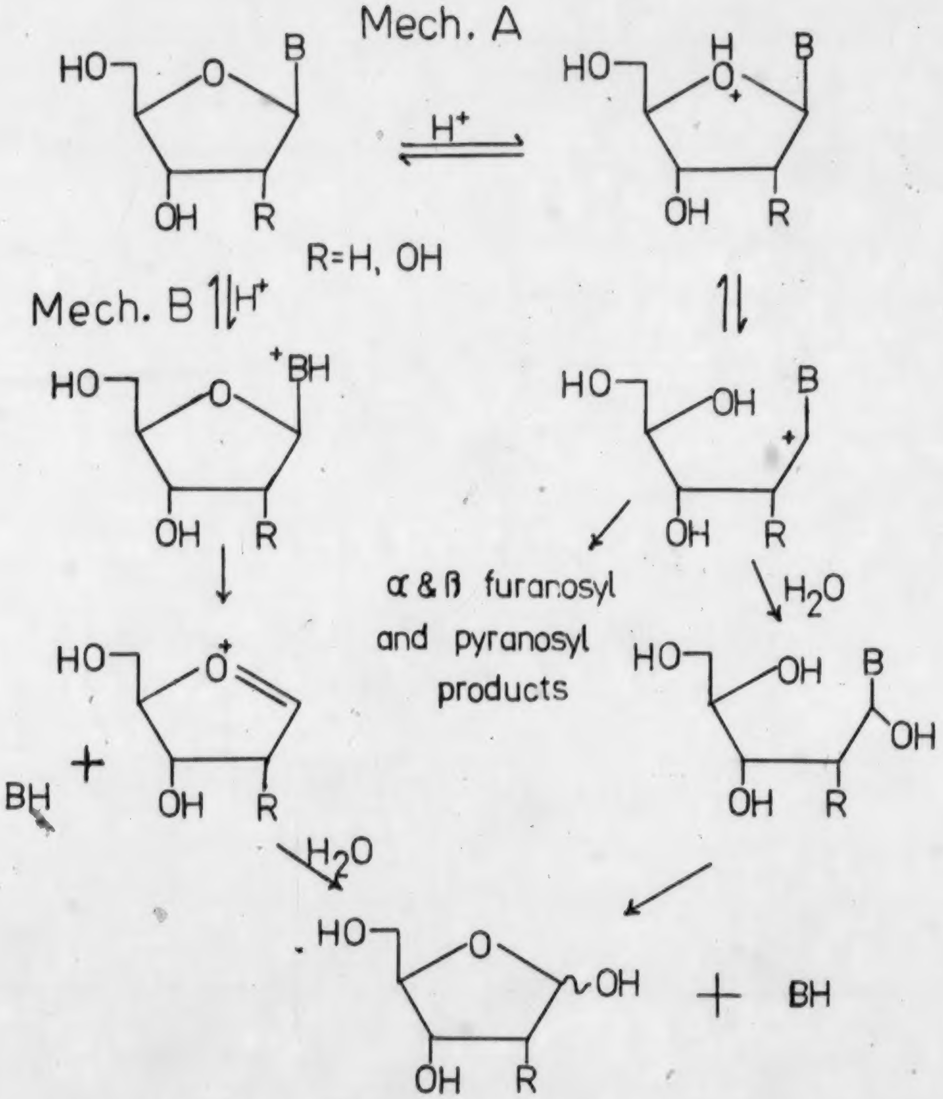
INTRODUCTION TO ACID-CATALYZED HYDROLYSIS OF THE BASE-GLYCOSYL BOND OF NUCLEOSIDES

A long time period elapsed between Kossel's [3] early acid hydrolyses of nucleic acids and the commencement of studies on the mechanism of hydrolysis of the glycosidic bond. (This introduction will be limited to the acid-catalyzed hydrolysis of this bond).

The first mechanism for this reaction was proposed by Kenner [90] and Dekker.[91] These workers suggested that the ring oxygen was protonated first, followed by transient formation of an unstable cationic Schiff base and subsequent hydrolysis. (See Figure 8, Mechanism A). Dekker [91] proposed that if the heterocyclic base could be protonated elsewhere, the ease of proton transfer to the sugar oxygen was an important factor. This explanation of the mechanism was based, in part, on the acid solvolysis of the simpler glycosylamines.[92]

Later workers, studying a variety of purine and pyrimidine nucleosides, showed that the mechanism of hydrolysis could be better explained by invoking an Al mechanism. (See Figure 8, Mechanism B).[93-101] These later workers proposed mono (and/or di) protonation of the base followed by base-sugar cleavage involving participation by the ring oxygen as the rate determining step and finally hydrolysis of the sugar moiety.

Figure 8


Mech. A
HO
O
B
H⁺
HO
H
O
B
OH
R
R=H, OH
Mech. B
H⁺
⁺BH
HO
OH
B
α & β furanosyl
and pyranosyl
products
H₂O
HO
O
BH
H₂O
OH
R
HO
OH
BH

Very recently, however, Cadet and Teoule [102] have reported that acid hydrolysis of thymidine and 2'-deoxyuridine results in formation of α and β furanosides and pyranosides. This can best be explained by reclosure of intermediates involved in the Kenner and Dekker mechanism. They also studied the acid hydrolysis of 5-bromo-2'-deoxyuridine and 2'-deoxycytidine but found only rupture of the N-glycosidic bond in harmony with the Al process (Mechanism B).

It is not surprising that with the vast variety of base structures, conditions of hydrolysis and different pKa's (adenosine, 3.45; cytidine, 4.22; guanosine, 1.6; uridine, ~0) that one mechanism of acid hydrolysis doesn't hold for all nucleosides. To date, in the case of purines, all hydrolyses can be satisfactorily accommodated by the Al mechanism.[95,97-100]

The relative stability of the glycosyl linkage towards acid is dependent on a variety of parameters. In general the stability of the glycosyl linkage in acid is in the order uracil (or thymine) > cytosine > adenine > guanine. In general purine nucleosides are much more reactive than pyrimidine nucleosides.[103]

Phosphorylation of the sugar (nucleotides) tends to stabilize the glycosyl linkage toward acid hydrolysis. Shapiro and Chargaff have shown that thymidine 5'-phosphate is more stable than thymidine under acid conditions.[104]

They also noted that thymidine-3',5'-diphosphate was even more stable under similar conditions. Studies of the stabilities in acid solution of 3',5'-cyclic phosphates of purine nucleosides and of their corresponding nucleoside 5'-phosphates reveal that the former are remarkably resistant to glycosyl cleavage compared to the latter. In the case of pyrimidine nucleoside 3',5'-cyclic phosphates (especially for uridine and thymidine), however, it was found that their 5'-nucleotides were more stable in acid.[105,106]

Montgomery and Thomas found that anomeric configuration in the case of adenine nucleosides (α or β) had only a slight affect on their stabilities in acid.[107] Leonard and Laursen found that the position of attachment of the base (adenine) to the anomeric position of the sugar affected the derivative's stability.[108] Adenosine was more stable to acidic hydrolysis conditions than 3-β-D-ribofuranosyladenine. Townsend and coworkers [109], after studying the acid-catalyzed hydrolysis of several 7-β-D-ribofuranosylpurines and 9-β-D-ribofuranosylpurines, found that the 7 isomer hydrolyzed faster than the 9 isomer (with the exception of guanine derivatives). Chemical ionization mass spectrometry [110] showed similar results when the relative glycosyl bond strengths of 7- and 9-β-D-ribofuranosylpurines were compared.

Modification of cytidine through base N-acylation[111] destabilized the nucleoside toward acidic hydrolysis. Base methylation of guanosine resulted in only small differences in the acid stability in weakly acidic solutions.[95]

In the study of the acid stability hydrolysis of a large variety of adenine nucleosides, Garrett found that the stability of the nucleoside increased with the increasing number of hydroxyl groups in the sugar.[100] the most important sugar hydroxyl was the 2'-OH, with adenosine 1000 times as stable as 2'-deoxyadenosine. The effect was independent of the aglycone (base).[95,112-113] The 3'-OH had a 5 to 7 fold stabilizing effect (when adenosine and 3'-deoxyadenosine were compared). Finally Garrett found that 2',3'-dideoxyadenosine solvolyzed 3 to 4 times faster than 2'-deoxyadenosine under identical conditions. Zoltewicz and coworkers attributed the stabilization of nucleosides by hydroxyl groups to an inductive effect which stabilizes the C-N bond against A 1 ionization.[107] Garrett [97] suggested that the hydroxyl groups may compete as proton acceptor sites for the approaching hydrogen ion and by coulombic effects repel the attack at the reaction site. In the same paper Garrett found that sugar configurational changes (riboside, arabinoside, and xyloside of adenine) had small effects on the solvolysis but that when the 2'-OH was "down" compared to adenine there was some stabilization. Also, base substitution effects (specifically methylation) gave only small changes in agreement with the results of Zoltewicz and coworkers.[95] The slightly enhanced stability observed for 2'-O-methyl adenosine was attributed to two possible effects by Garrett.[100] He reasoned that either it was due to a steric effect (hindering solvation of the developing carbonium ion) or to the fact that 2'-O-methyl adenosine could not hydrogen bond with the base as a 2'-OH could. This hydrogen bonding might then reduce the inductive withdrawing effect of the 2'-OH (as compared to the 2'-OCH_3). Reese and coworkers also noticed enhanced stability of both 2'-O-methyl adenosine and 3'-O-methyl adenosine relative to adenosine in acid.[114] Using their hydrolysis conditions (1.0 N HCl at 41°), they found a considerable improvement in the half-life stability for 2'-O-methyl adenosine (19.6 h) and a lesser improvement for 3'-O-methyl adenosine (12.8 h) compared with adenosine (7.9 h).

Besides the stabilization of phosphates already mentioned, several authors have noticed the great stability of certain sugar substituted nucleosides. Michelson noted enhanced stability exhibited by O-acetyl-2'-deoxyguanosine.[115] Brown and coworkers noted that the 3',5'-O-diacetyl derivative of 2'-O-p-toluenesulfonyl adenosine was deacylated without glycosyl cleavage when treated with refluxing methanolic HCl for 12 h.[116] Ulbricht reported that 2'-O-p-nitrobenzenesulfonyl adenosine was stable under conditions which hydrolyzed adenosine.[117] Ikehara noted improved acid stability with 3'-O-p-nitrobenzenesulfonyladenosine and attributed it to interaction between the sugar substituent and the base.[118]

Several workers have made use of the acid stabilization effect of certain sugar substituents to perform reactions employing acidic conditions under which an unprotected nucleoside would not survive. The reaction by Brown[116] with 2'-O-p-toluenesulfonyl adenosine has already been noted. Fox and coworkers made use of the p-nitrobenzoyl protecting group to stabilize uridine to fuming nitric and concentrated sulfuric acids in a nitration reaction.[119] Robins and Robins detritylated 3'-O-p-toluenesulfonyl-5'-O-trityl-2'-deoxyadenosine in 80% acetic acid under conditions which hydrolyzed 2'-deoxyadenosine.[120]

Also, Robins and Basom protected 2'-deoxyinosine from acidic cleavage by blocking the sugar with trifluoroacetyl groups.[121]

Thus it can be seen that substitution of the sugar with electron withdrawing groups offers a method of protection of the nucleoside from acidic hydrolysis of the glycosidic bond.

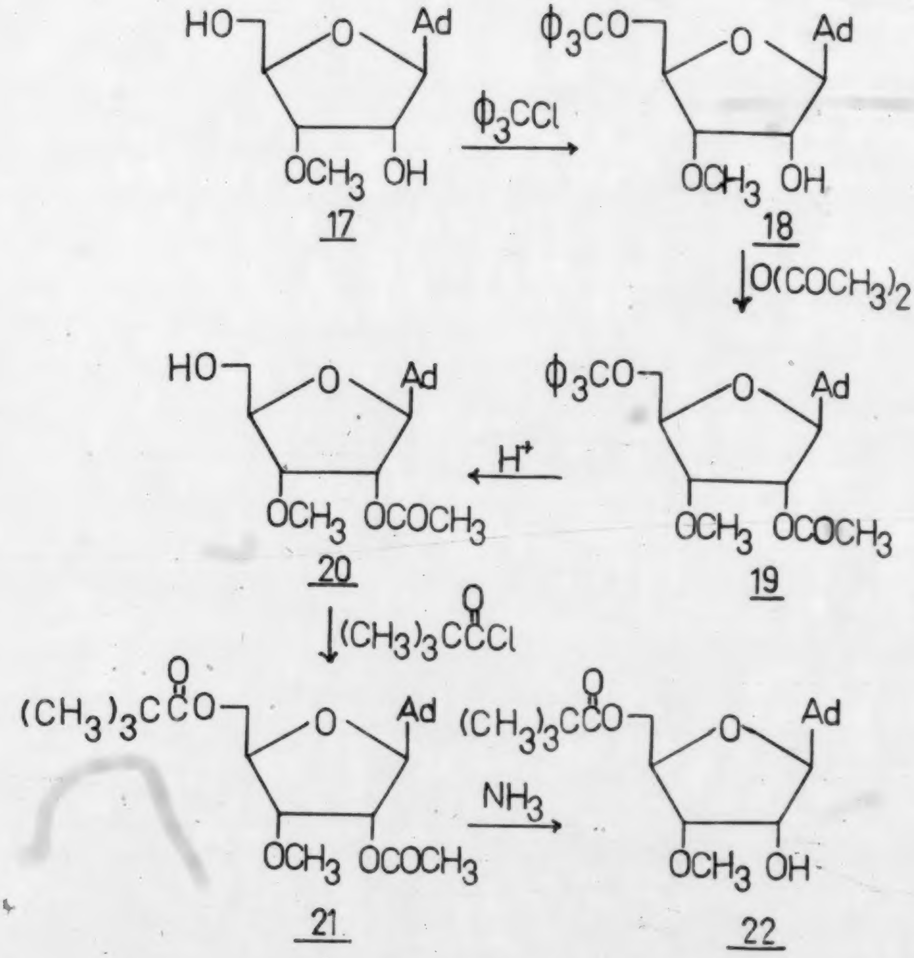
DISCUSSION AND RESULTS

SYNTHESIS OF 2'-DEOXY ADENOSINE COMPOUNDS

In order to investigate reactions at the 2' position of adenosine it was necessary to block the 3'- and 5'-hydroxyl groups of the sugar. It was foreseen that certain types of reactions involve acidic conditions. Thus, to avoid complications acid stable blocking groups were chosen.

The facile methylation of the 2'- and 3'-hydroxyl groups using diazomethane with stannous chloride as catalyst [122] to give (17) (see Figure 9) offered a very stable blocking group for the 3' position. Also, steric and electronic disruptions of the 2'-hydroxyl would be minimized with the 3'-methyl ether. Attempts to block the 5'-hydroxyl selectively with the bulky pivalyl group failed; as did attempts to selectively deblock the 2',5'-dipivalyl derivative. Thus, a longer synthetic route was required. This involved selective blocking of the primary 5'-hydroxyl with the triphenylmethyl (trityl) group, to give (18), followed by selective acetylation (low temperature) of the 2'-hydroxyl to give (19). This compound was detritylated in acid to yield (20) which was pivalylated at the 5' position to give (21). Some base pivalylation occurred but treatment with concentrated aqueous NH_3 selectively removed the base pivalyl and the 2'-O-acetyl

Figure 9


HO
Ad
O
OCH3
OH
17
Φ3CCl
Φ3CO
18
O(COCH3)2
19
OCOCH3
H+
20
(CH3)3CCCl
(CH3)3CCO
21
NH3
22

to give the desired product (22). This procedure involved several steps but with the exception of the original tritylation all other steps proceed in good yield to easily crystallized products with a minimum of workup.

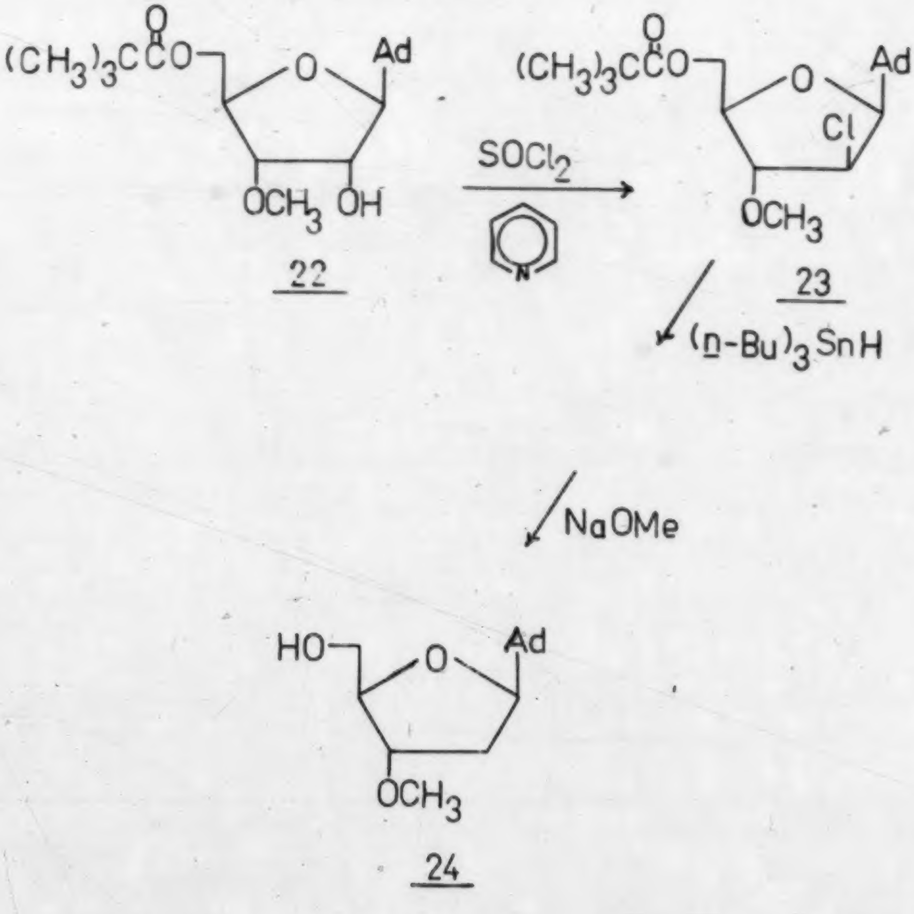
The introduction of tri-n-butyltin hydride as a reducing agent for sugar chlorides and bromides [123,124] made the conversion of these compounds (previously very difficult to reduce) to their respective deoxy compounds easily possible. Holý [59-61] and other workers [125] made use of this reagent in the syntheses of pyrimidine 2'-deoxynucleosides. Very recently Corey [126] has reported a modification that greatly increases the applicability of this reaction. Only the choice of a successful chlorinating agent remained to complete the sequence. Thionyl chloride had been used to chlorinate the 5' position of nucleosides.[127,128] Also, Vilsmeier - Haack conditions had been used with either N,N'-dimethylformamide (DMF) or hexamethylphosphoramide (HMPA) and thionyl chloride to halogenate the 5' and 3' positions of nucleosides.[129-133] Hogenkamp [133] claimed that a 2'-chloro-2'-deoxynucleoside was formed from xylosyladenine under these conditions. He suggested that a cyclic intermediate involving the 3' and 5' position of xylosyladenine had formed with HMPA and that this intermediate protected these positions from further attack. He offered as proof the fact that his chloro product was unreactive with cob (I) alamin and this vitamin model was known to be unreactive towards secondary alkyl halides.

Hogenkamp's procedure was repeated and the chloro product was isolated. This compound appeared to be the 5'-chloro-5'-deoxyxylosyladenine rather than the postulated 2'-substituted product. Proof of this fact included the mass spectrum, which had characteristic fragmentations that could only occur for a 5'-chloro product (see discussion on mass spectra) and the nuclear magnetic resonance spectrum which showed the largest shifts (from xylosyladenine) for the 5', 5" and 4' protons of the sugar. When this product was heated, a new compound was formed which showed shifts for the $H^{1'}$ ($\delta = 6.30$), H^2 and H^8 ($\delta = 8.47, 8.70$) protons characteristic of N-3 to C-5' cyclonucleoside formation.[134,135] Electrophoresis in a borate buffer and paper chromatography using a borate system indicated that the cis-1,3-diol structure of xylosyladenine (3' and 5' positions) had been changed. This would not have been expected if the compound was 2' substituted (see experimental).

Considerable research into the decomposition of secondary alkyl chlorosulfites[136-140] has shown that these compounds can rearrange via an SN_2 or an SN_1 (ion pair collapse) mechanism giving inversion or retention of the resulting chloro compound. The mechanism is affected by solvent changes or by heating in absence of solvent.

When 22 was treated with thionyl chloride, in hot pyridine, a product was isolated which proved to be the 2'-chloro compound (23). This product was obtained in ~20% yield. Numerous attempts to improve this yield by varying solvents, quantities of reagents, temperatures and by using Lewis acids [141] failed. Nucleosidic products isolated were unreacted starting material and 23. The reaction rapidly turned black on heating and a black precipitate remained after workup. Neither mild nor vigorous acid or base hydrolysis of this amorphous black solid yielded any detectable nucleosidic or heterocyclic products (such as adenine). Thus it seemed that the reaction was accompanied by extensive decomposition or polymerization of starting material. The chlorination reaction was assumed to go with inversion of configuration (SN_2) at the 2' position with pyridine as solvent.[139] (Proof of inversion of configuration in a similar reaction follows). This reaction would then give 9-(2-chloro-2-deoxy-3-O-methyl-5-O-pivalyl-β-D-arabinofuranosyl)adenine (23). (See Figure 10). The low yield of this reaction again illustrates the well known difficulty in performing nucleophilic displacements at C-2 of sugars[142] and especially C-2' of adenosine derivatives.[67,135,143]

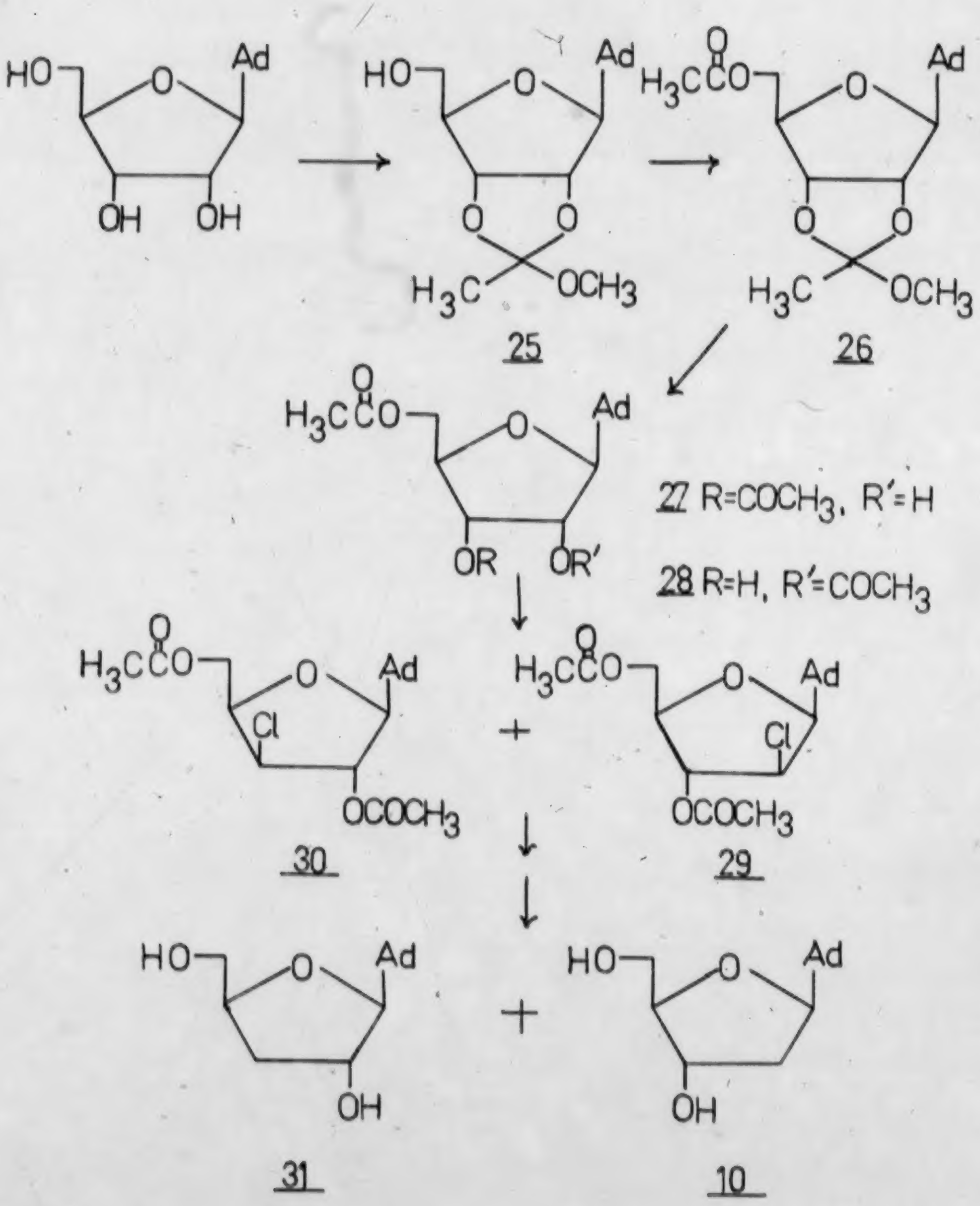
Figure 10


(CH3)3CCO-
Ad
O
OCH3
OH
22
SOCl2
Cl
23
(n-Bu)3SnH
NaOMe
HO
24

In reference 67 this point is illustrated by the difficulty in forming the ribo-epoxide by intramolecular nucleophilic attack involving 2'-chloro-2'-deoxyarabinofuranosyladenine. Compound 23 was dechlorinated using standard conditions for the tri-*n*-butyltin hydride reduction. After deblocking, 9-(2-deoxy-3-*O*-methyl-β-*D*-ribofuranosyl)-adenine (24) was obtained in excellent yield.

2'-Deoxyadenosine (10) was synthesized in a similar manner. In order to maximize yields in this reaction sequence (see Figure 11) unpurified material was carried through until the final isolation. Adenosine was converted into 9-(2,3-*O*-methoxyethylidene-β-*D*-ribofuranosyl)adenine (25) by a modification [144] of the procedure reported by Reese and coworkers.[145] The crude material was then selectively acetylated (5'-hydroxyl) using acetic anhydride and 4-dimethylaminopyridine as catalyst to give 9-(5-*O*-acetyl-2,3-*O*-methoxyethylidene-β-*D*-ribofuranosyl)-adenine (26). This material was converted in acid to a mixture of 9-(3,5-di-*O*-acetyl-β-*D*-ribofuranosyl)adenine (27) and 9-(2,5-di-*O*-acetyl-β-*D*-ribofuranosyl)adenine (28) by a known procedure [145] in a ratio of ~2 to 1, respectively. Thus the majority of this mixture has a free 2'-hydroxyl group for further reaction. Treatment of this mixture with thionyl chloride in hot pyridine gave a mixture of 9-(2-chloro-2-deoxy-3,5-di-*O*-acetyl-β-*D*-arabinofuranosyl)adenine (29) and 9-(3-chloro-3-deoxy-

Figure 11


HO
Ad
O
OH
OH
25
H_3C
OCH_3
26
H_3CCO
27 R=COCH_3, R'=H
28 R=H, R'=COCH_3
OR
OR'
Cl
OCOCH_3
30
29
31
10

2,5-di-O-acetyl-β-D-xylofuranosyl)adenine (30) in a ratio of ~3 to 2, respectively. To maximize this yield, the reaction was continued until ~60% of the starting material had disappeared (i.e. 40% remaining). Increasing the duration of the reaction failed to increase product yields and merely resulted in more extensive decomposition. Under these conditions the reaction proceeded in a fair yield. The crude chlorinated mixture was reduced with tri-n-butyltin hydride (which decreased the colour significantly) and was deblocked with base. The resulting mixture was easily separated by the procedure first outlined by Dekker [146], using anion exhange on a Dowex 1-X2 (OH^-) resin column with varying concentrations of aqueous alcohol as eluant. This column separation resulted in isolation of pure 2'-deoxyadenosine (10) and 3'-deoxyadenosine (31) in a ratio of ~3 to 2, respectively. The overall yield of 2'-deoxyadenosine from adenosine was 16% (26.6% based on recovered starting material).

It is interesting that in the preparation of both 10 and 24, the free radical reduction with tri-n-butyltin hydride proceeds in excellent yield when compared with the nucleophilic displacement in the reaction with thionyl chloride. These results would suggest that the best method to functionalize the 2' position of adenosine would be through a radical mechanism.

The trans configurations at C-2' and C-3' in the chloro compounds 29 and 30 were established by isolation of each after deblocking and chromatography, followed by comparison with known compounds. Also, both chloro compounds 29 and 30 were converted to the ribo epoxide in base (again the 2' chloro product required more vigorous conditions).

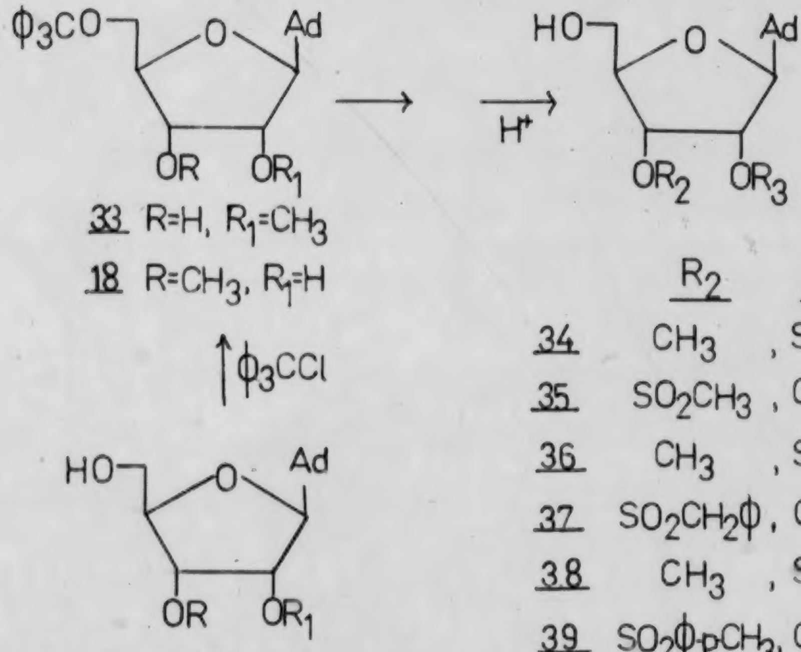
SYNTHESIS OF 2' AND 3' SULFONYL AND CARBOXYLIC ESTERS OF ADENOSINE

A series of sulfonyl and carboxylic esters were synthesized in order to study their physical properties (cd, uv, nmr, ms) and rates of acid-catalyzed hydrolysis. This investigation was initiated to resolve certain ambiguities in the literature.

The 2'- and 3'-O-methyladenosines (32 and 17 respectively) were chosen as starting materials for reasons outlined in the previous section.

Both of these nucleoside ethers were selectively tritylated at the 5' position to give 18 and 9-(2-O-methyl-5-O-triphenylmethyl-β-D-ribofuranosyl)adenine (33). Preparation of the blocked alkyl and aryl sulfonyl compounds was achieved using standard procedures [143,147] followed by acidic removal of the trityl group to yield the desired compounds (see Figure 12). Compounds 9-(2-O-methanesulfonyl-3-O-methyl-β-D-ribofuranosyl)adenine (34), 9-(3-O-methanesulfonyl-2-O-methyl-β-D-ribofuranosyl)adenine (35), 9-(2-O-benzylsulfonyl-3-O-methyl-β-D-ribofuranosyl)adenine (36) and 9-(3-O-benzylsulfonyl-2-O-methyl-β-D-ribofuranosyl)-adenine (37) were synthesized in good yields without formation of by-products. Compounds 9-(3-O-methyl-2-O-p-toluenesulfonyl-β-D-ribofuranosyl)adenine (38), 9-(2-O-methyl-3-O-

Figure 12


ϕ_3CO
O
Ad
OR
OR_1
H^+
HO
O
Ad
OR_2
OR_3
ϕ_3CCl
HO
O
Ad
OR
OR_1

33 $R=H, R_1=CH_3$
18 $R=CH_3, R_1=H$

32 $R=H, R_1=CH_3$
17 $R=CH_3, R_1=H$

	R_2	R_3
34	CH_3	SO_2CH_3
35	SO_2CH_3	CH_3
36	CH_3	$SO_2CH_2\phi$
37	$SO_2CH_2\phi$	CH_3
38	CH_3	$SO_2\phi$-p-CH_3
39	$SO_2\phi$-pCH_3	CH_3
40	CH_3	$SO_2\phi$-p-NO_2
41	$SO_2\phi$-p-NO_2	CH_3
42	CH_3	$SO_2\phi$-p-NH_2
43	$SO_2\phi$-p-NH_2	CH_3
44	CH_3	SO_2CF_3
45	SO_2CF_3	CH_3

p-toluenesulfonyl-β-*D*-ribofuranosyl)adenine (**39**), 9-(3-*O*-methyl-2-*O*-*p*-nitrobenzenesulfonyl-β-*D*-ribofuranosyl)adenine (**40**) and 9-(2-*O*-methyl-3-*O*-*p*-nitrobenzenesulfonyl-β-*D*-ribofuranosyl)adenine (**41**) were prepared in moderate yields with formation of small amounts of other products (possibly base substituted derivatives).

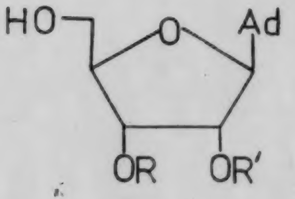
It is interesting to note that the alkyl sulfonyl compounds reacted very rapidly (~1 h) and in generally better yields when compared to the aryl sulfonyl compounds (reactions as long as 1 week). Moffatt and coworkers[148] have since reported a facile method of synthesis of compounds such as 2'-*O*-*p*-toluenesulfonyladenosine from adenosine using activating organotin derivatives.

Compounds 9-(2-*O*-*p*-aminobenzenesulfonyl-3-*O*-methyl-β-*D*-ribofuranosyl)adenine (**42**) and 9-(3-*O*-*p*-aminobenzenesulfonyl-2-*O*-methyl-β-*D*-ribofuranosyl)adenine (**43**) were prepared by hydrogenation of the corresponding *p*-nitrobenzenesulfonyl compounds **40** and **41**, respectively, using palladium on charcoal. These reductions were selective and the only complication was a tendency of the products to decompose upon heating.

Compounds 9-(3-*O*-methyl-2-*O*-trifluoromethanesulfonyl-β-*D*-ribofuranosyl)adenine (**44**) and 9-(2-*O*-methyl-3-*O*-trifluoromethanesulfonyl-β-*D*-ribofuranosyl)adenine (**45**) were prepared in low yield using trifluoromethanesulfonic anhydride. Several attempts were made to improve the yields of these reactions with only moderate success. Compound 45 proved to be extremely sensitive and decomposed even when kept at low temperatures with exclusion of moisture. This compound may decompose through the cyclonucleoside with bond formation between N3 and C-3' similar to the decomposition of riboepoxides on heating.[135,149] Part of the difficulty in preparation of the trifluoromethanesulfonyl compounds is certainly due to the extremely reactive nature of triflate esters.[150] The following abbreviations will be used: mesyl for methanesulfonyl, besyl for benzylsulfonyl, tosyl for p-toluenesulfonyl, nisyl for p-nitrobenzenesulfonyl, aminosyl for p-aminobenzenesulfonyl and triflyl for trifluoromethanesulfonyl.

Compounds 9-(3-O-benzyl-β-D-ribofuranosyl)adenine (46) and 9-(2-O-benzyl-β-D-ribofuranosyl)adenine (47) were prepared by the procedure of Broom[151] (see Figure 13).

Figure 13

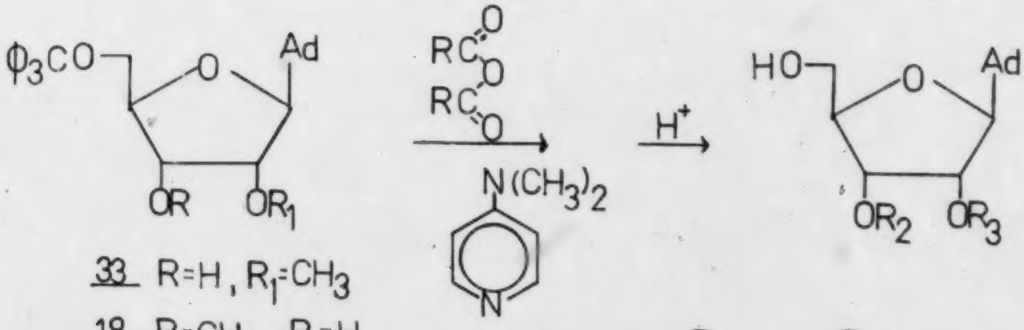


46 R'=H, R=CH$_2\phi$

47 R'=CH$_2\phi$, R=H

The esters 9-(2-*O*-*p*-methoxyphenylacetyl-3-*O*-methyl-β-*D*-ribofuranosyl)adenine (**48**), 9-(3-*O*-*p*-methoxyphenylacetyl-2-*O*-methyl-β-*D*-ribofuranosyl)adenine (**49**), 9-(2-*O*-*p*-methoxybenzoyl-3-*O*-methyl-β-*D*-ribofuranosyl)adenine (**50**) and 9-(3-*O*-*p*-methoxybenzoyl-2-*O*-methyl-β-*D*-ribofuranosyl)adenine (**51**) were prepared by a procedure used in this laboratory [152] (see Figure 14). The anhydride of the acid was prepared using *N*,*N*'-dicyclohexylcarbodiimide and this solution was added to a solution containing **18** or **33** and 4-*N*,*N*-dimethylaminopyridine (the reaction would not proceed without this catalyst). After acidic removal of the trityl group, the desired compound was isolated. It is again of interest that the alkyl anhydrides (*p*-methoxyphenylacetyl) reacted much more rapidly (~5 min) than the aromatic anhydrides (*p*-methoxybenzoyl in about 1 to 7 days).

Figure 14


ϕ_3CO
O
Ad
OR
OR$_1$
RC(=O)
O
RC(=O)
N(CH$_3$)$_2$
N
H$^+$
HO
O
Ad
OR$_2$
OR$_3$

33 $R=H$, $R_1=CH_3$

18 $R=CH_3$, $R_1=H$

	R_2	R_3
48	CH_3	, $COCH_2\phi$-p-OCH_3
49	$COCH_2\phi$-p-OCH_3,	CH_3
50	CH_3	, $CO\phi$-p-OCH_3
51	$CO\phi$-p-OCH_3	, CH_3

ULTRAVIOLET AND CIRCULAR DICHROISM SPECTRA

In recent years there have been numerous reports of base, sugar-substituent overlap in nucleosidic compounds. Ikehara claimed that such an overlap was observed in 3'-O-p-nitrobenzenesulfonyladenosine.[119] By subtracting the absorption spectrum of this compound from the sum of the absorption spectra of adenosine and 1,2-O-isopropylidene-3-O-p-nitrobenzenesulfonyl-D-xylofuranose, he calculated a 13% hypochromicity at 257 nm. He quoted this as proof of base, sugar-substituent overlap and suggested that this accounted for the unusual stability of this compound against bromination and acid cleavage. The following discussion and the discussion on rates of acid hydrolysis will dispute these conclusions.

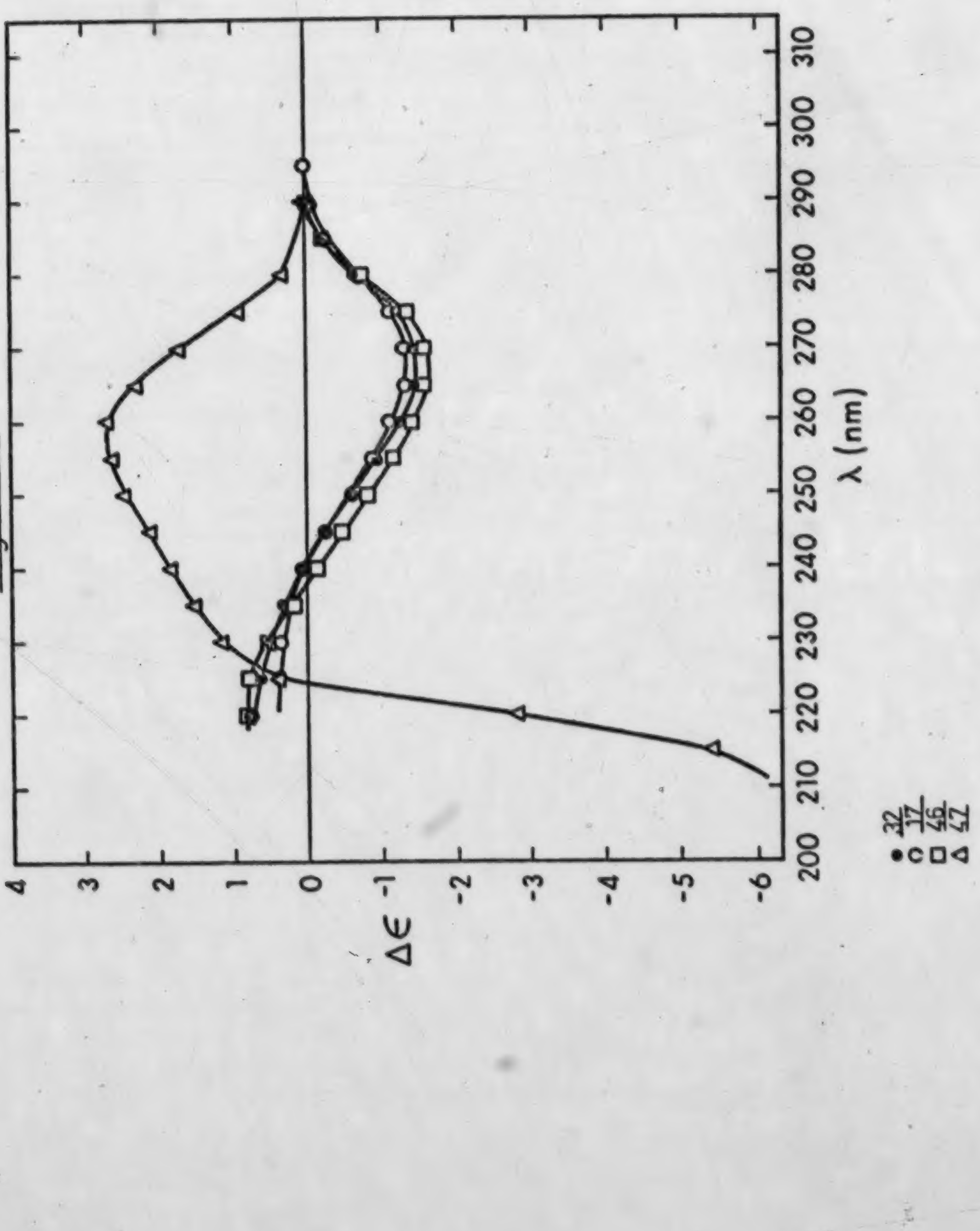
In later papers, Ikehara studied base, sugar-substituent overlap in 8-bromo-2'-O-triisopropylbenzenesulfonyladenosine and also showed that no overlap was present in the 8-bromo-3'-O-triisopropylbenzenesulfonyladenosine isomer. Intramolecular nuclear Overhauser effects [153] and X-ray analysis [154] confirmed overlap in the 2'-isomer and a nonstacked form for the 3'-isomer. The uv spectra of these compounds, however, were unusual.[155] Whereas both the 5'-O-acetyl and 5'-O-trityl derivatives of the above compounds showed hypochromicity for the 2'-isomer (compared with the 3'-isomer), the parent 2'-isomer (8-bromo-2'-O-triisopropylbenzenesulfonyladenosine) showed no uv hypochromicity relative to the 3'-isomer.

Broom observed base, 2'-sugar-substituent overlap in a variety of 2'-O-benzylated nucleosides. Whereas the 3'-isomers gave normal extinction coefficients (3'-O-benzyladenosine at pH 7, ϵmax 15,000), the 2'-isomers showed marked hypochromicities (2'-O-benzyladenosine at pH 7, ϵmax = 13,000).[151] Broom noted that stacking of the base and the 3'-O-benzyl group was impossible by examination of models. Pfleiderer has very recently reported enhancement of the cd of 2'-O-benzyl ethers of uridine compounds (compared to the cd spectra of the 3'-O-benzyl isomers) and has postulated base, sugar-substituent overlap.[156]

Examination of the uv and cd spectra of compounds synthesized in this thesis provided interesting structural vs spectral correlations. It should be noted here that great care was taken in the determination of the cd (and uv) spectra. Most cd spectra were repeated at several different concentrations.[19] The instrument used to obtain the cd spectra (see experimental section) recorded the photomultiplier voltage at each wavelength as the spectrum was recorded. Only the portion of the spectrum which had this voltage at acceptable levels (to prevent rotatory artifacts) and gave a reasonable signal to noise ratio was plotted in the following cd figures. All determinations were run in spectral grade methanol unless otherwise stated.

Both 2'-O-methyl- (32) and 3'-O-methyl-adenosine (17) had cd spectra (see Figure 15) which exhibit the expected negative Cotton effect [19] with a minimum at λ ~270 nm. The magnitudes of the Cotton effects are also consistent with adenosine derivatives in the anti conformation.[19] When these spectra are compared with the cd spectra of 2'-O-benzyl- (47) and 3'-O-benzyladenosine (46) (Figure 15) some interesting differences are apparent. Whereas 46 has a cd spectrum almost exactly the same as that of 32 and 17 and shows no uv hypochromicity, 47 has two very different Cotton effects. It is apparent from the uv hypochromicity of 47 that this isomer has overlap between the adenine base and the 2'-O-benzyl sugar substituent.[151] The cd spectrum shows the effect of this overlap. In the cd spec-spectrum of 47 there is a large positive Cotton effect with a maximum at λ ~260 nm which indicates that the nucleoside conformation has changed; most likely by a rotation of the adenine base to accomodate overlap with the sugar substituent. The Cotton effect at 260 nm is attributed to the adennine base since the benzyl group has no significant uv absorption in this region. At λ ~210 nm a very large enhanced Cotton effect occurs indicating base, sugar-substituent overlap. It should be noted that although the extremum of this large Cotton effect was not recorded due to noise interference in the cd, the corresponding ord spectrum did show one of the ord extrema at λ = 217 nm.

Figure 15


Δε
4
3
2
1
0
-1
-2
-3
-4
-5
-6
200
210
220
230
240
250
260
270
280
290
300
310
λ (nm)
● 32
○ 17
□ 46
Δ 47

The cd spectra of the 2'-O-triflyl-(44), 3'-O-triflyl-(45), 2'-O-mesyl-(34) and 3'-O-mesyl-(35) 2'(3')-O-methyladenosine compounds had almost identical cd spectra to those of 17 and 32 (see Figure 16). This is as expected with the non-aromatic sugar substituents that cannot interact with the adenine base by π-π overlap. This lack of base, sugar-substituent overlap is also confirmed by their relative uv extinction coefficients (see experimental section) and nmr spectra (see discussion on nmr).

The cd spectrum of 2'-O-tosyl-3'-O-methyladenosine (38) (see Figure 17) exhibits an enhancement in the magnitude of the cd spectrum, at low wavelengths relative to that of the 3'-O-tosyl isomer (39). This again indicates stacking of the base and the 2'-O-tosyl group. The strength of the interaction between the base and the sugar in 38 was demonstrated by the small effect that an increase in temperature had on the cd spectrum. For example, the large Cotton effect at λ = 212 nm was decreased by only ~4% upon increasing the temperature from 25° to 60°. This base, sugar-substituent overlap also gives a large hypochromicity in the uv spectrum (see experimental section) and markedly affects the nmr chemical shifts for this compound (see discussion on nmr). The uv hypochromicity of a 2'-O-tosyl substituent was also noted by Moffat and coworkers.[148] The cd spectrum of 38 is more complex since the sugar tosyl substituent has a uv maximum at λ ~ 230 nm. Thus, there

Figure 16

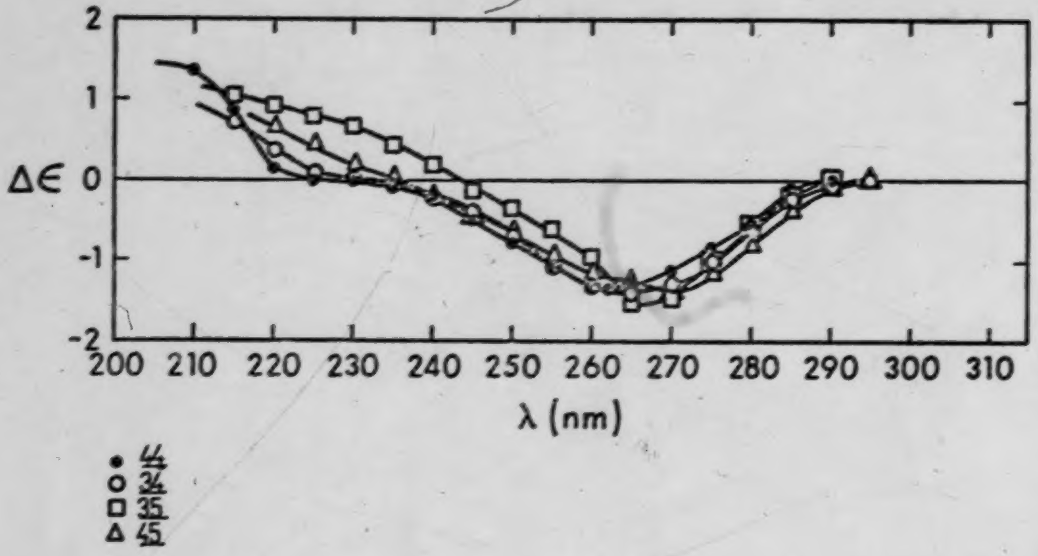
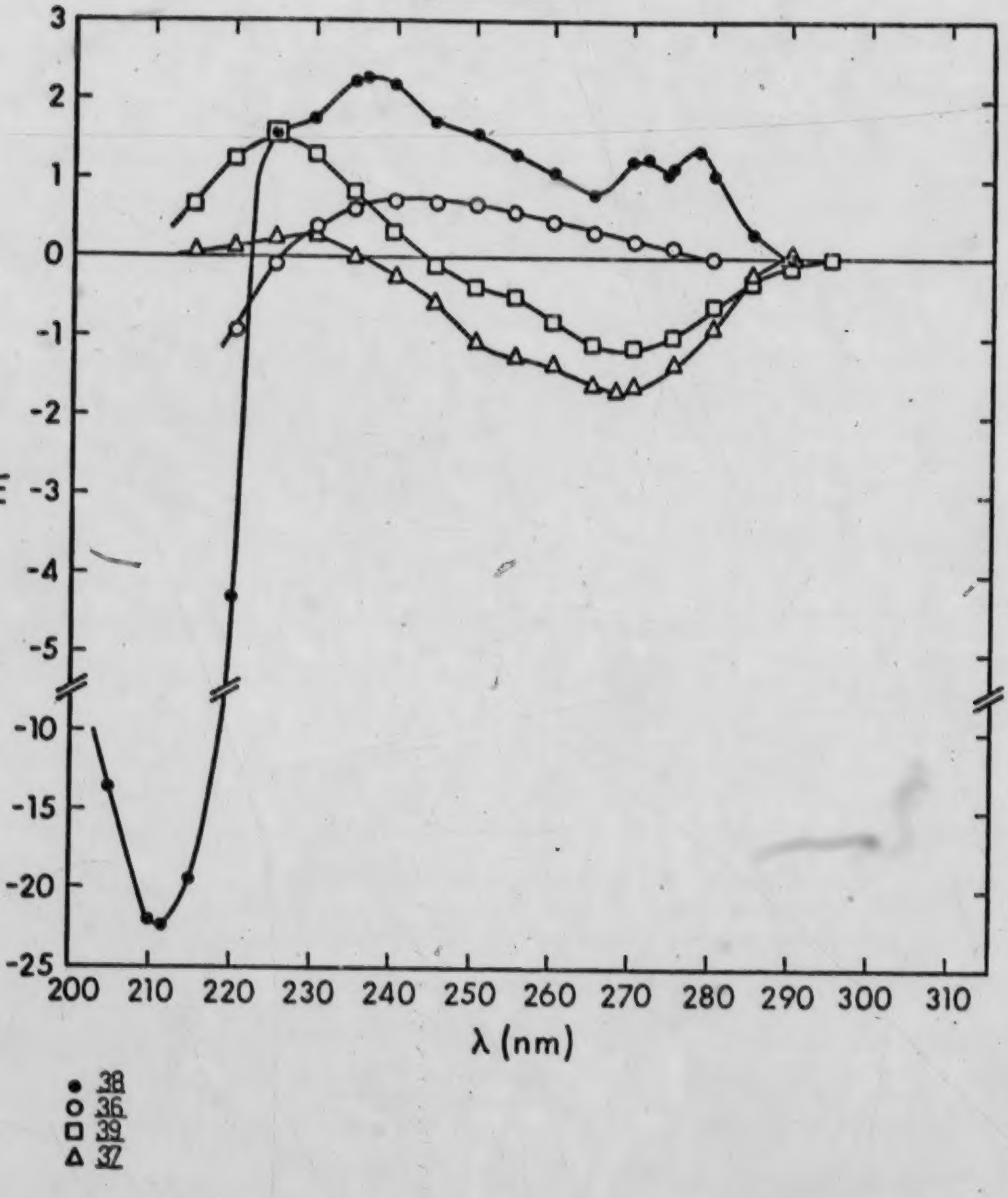

ΔЄ
2
1
0
-1
-2
200
210
220
230
240
250
260
270
280
290
300
310
λ (nm)
● 44
○ 34
□ 35
Δ 45

Figure 17


Δϵ
3
2
1
0
-1
-2
-3
-4
-5
-10
-15
-20
-25
200
210
220
230
240
250
260
270
280
290
300
310
λ (nm)
● 38
○ 36
□ 39
△ 37

is overlap of Cotton effects from the base (adenine) and the tosyl group in this region.

A comparison of the cd spectra of 2'-O-besyl-(36) and 3'-O-besyl-(37) 2'(3')-O-methyl adenosines again shows a difference in the Cotton effects (see Figure 17). In this case, however, the cd spectrum of 36 shows only a small low wavelength enhancement, if any, but the first Cotton effect ($\lambda \sim 250$ nm) is positive. This Cotton effect presumably arises from the adenine base which indicates that some change in conformation of the nucleoside has occurred (again most likely a torsional rotation of the base). The uv spectrum of 36 shows a smaller hypochromicity than that of 38 or 47 and no marked shifts occur in the nmr spectrum (see discussion on nmr). Thus, it appears that there is some base, sugar-substituent overlap, possibly in equilibrium with a "non-stacked" form, but certainly the overlap is not as strong as with some other derivatives noted in this discussion. This is probably due to the greater distance between the base and the aromatic sugar substituent owing to the extra methylene group. Leonard and coworkers [157] have shown that stacking interactions between two adenine rings decrease with increasing separation of the bases. This separation was studied by joining the adenine rings through methylene bridges. The optimum stacking interaction was obtained with a linear bridge of three methylene groups.[157]

The cd spectra of 2'-O-nisyl- (40), 3'-O-nisyl- (41), 2'-O-aminosyl- (42) and 3'-O-aminosyl—(43) 2'(3')-O-methyl adenosines are more complex. Both the base and the sugar substituents have uv maxima in the same region which results in overlapping Cotton effects (see Figure 18). In general though, the 2'-isomers have greatly enhanced Cotton effects when compared with the 3'-isomers. This would suggest base, sugar-substituent overlap, as would the hypochromicities observed in the uv spectra and chemical shifts in the nmr spectra shown by the 2'-isomers (see experimental and discussion on nmr, respectively).

In acidic solutions, the cd spectra change radically, as expected, since at least the adenine (and in the case of aminosyl, both) chromophore(s) is protonated (see Figure 19). This Figure shows the spectra of 38 and 42 run in a mixture of MeOH : H_2O (1:4) at pHs 6.2 and 1.1. Here an anomaly arises. Whereas the uv spectrum of 38 at acidic pH shows hypochromicity and a fairly large cd effect is observed (although not as large as at higher pH), the uv spectrum of 42 at acidic pH shows no hypochromiticy but a very large cd effect is present. At a pH of 1.1, both the base and the 2'-aminosyl substituent of 42 should be protonated. This would result in a large electrostatic repulsion which explains the lack of uv hypochromicity. The large cd effect must arise by some other enhancement mechanism, such as restricted rotation of either (or both)

Figure 18

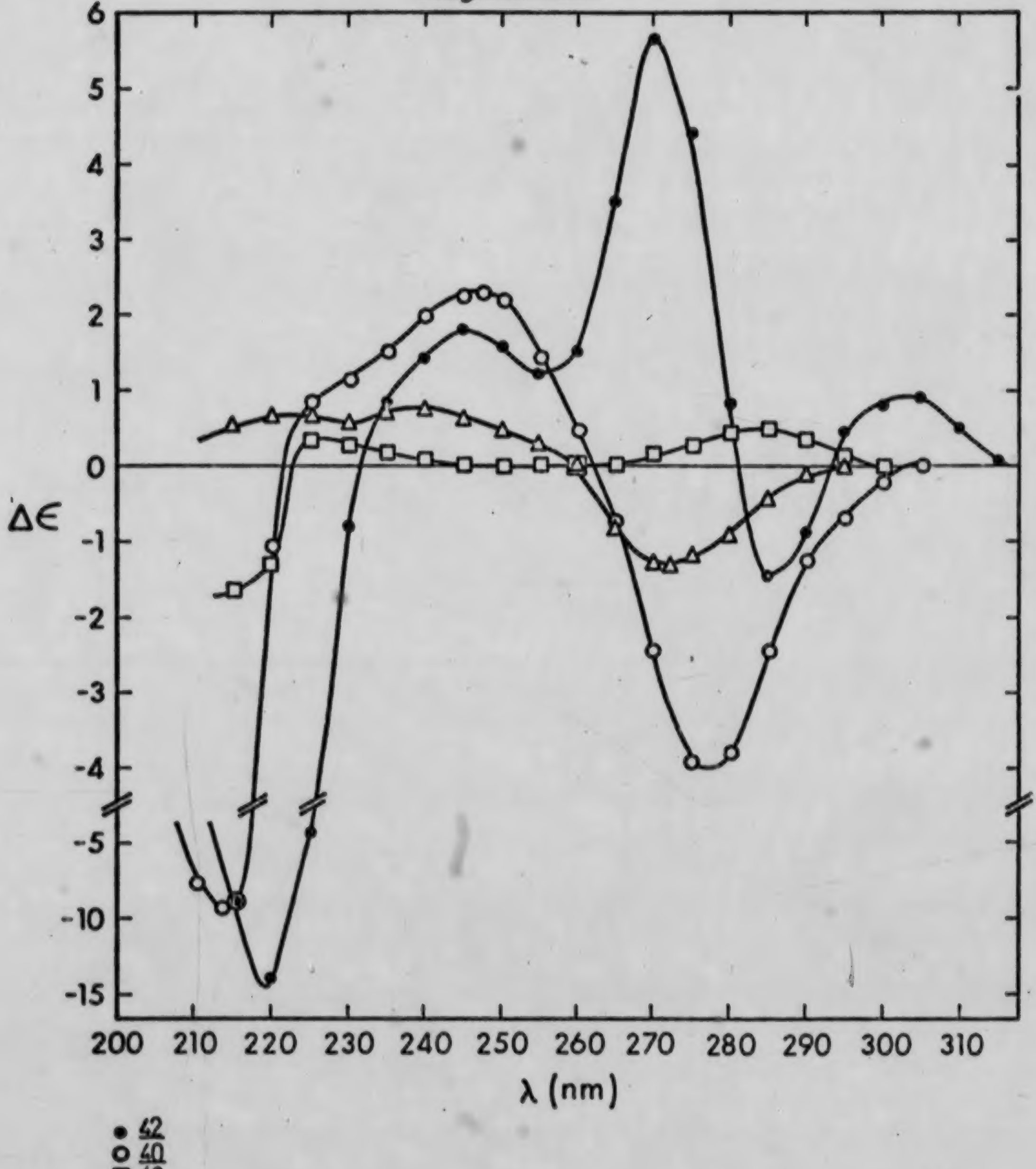
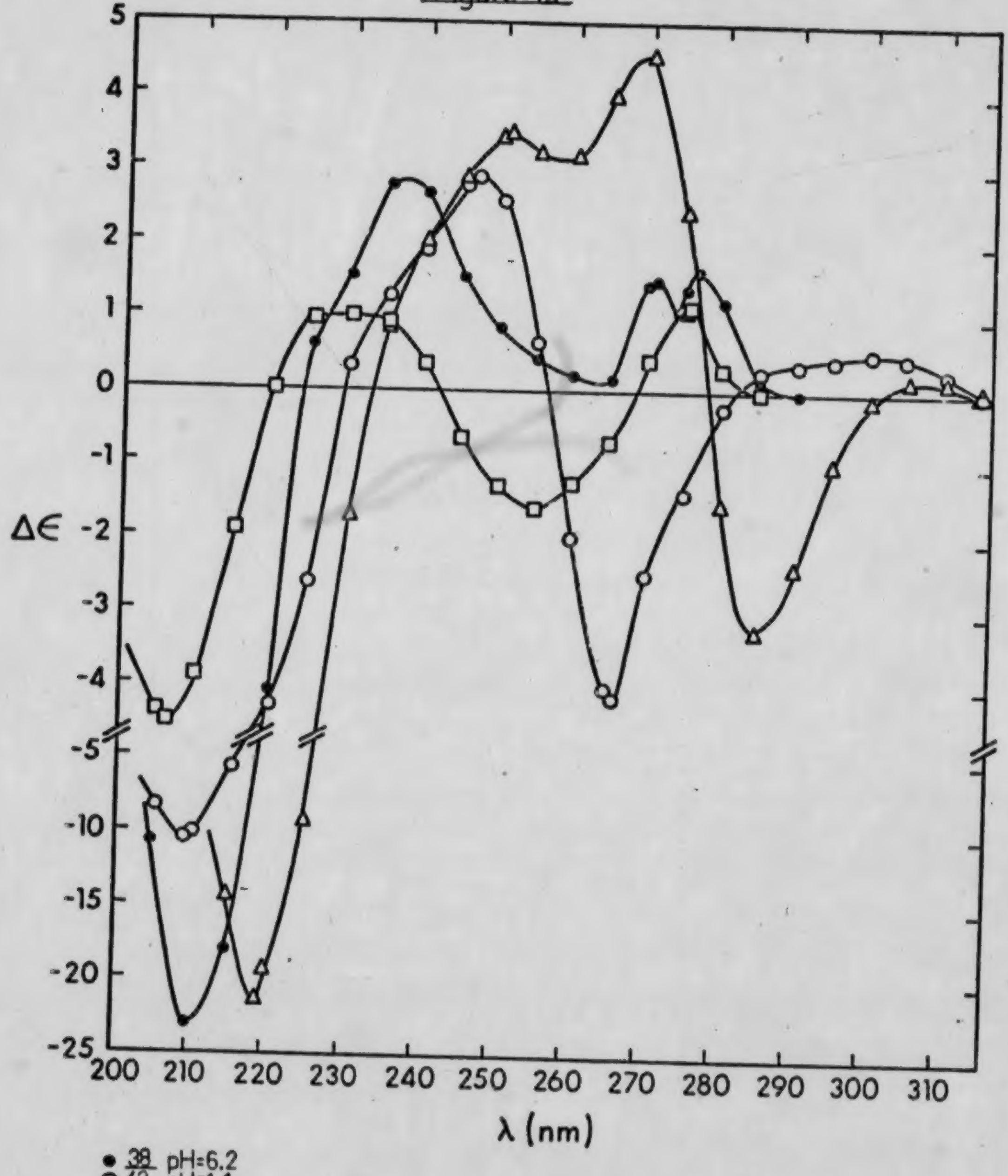

Δϵ
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-10
-15
200
210
220
230
240
250
260
270
280
290
300
310
λ (nm)
● 42
○ 40
□ 43
Δ 41

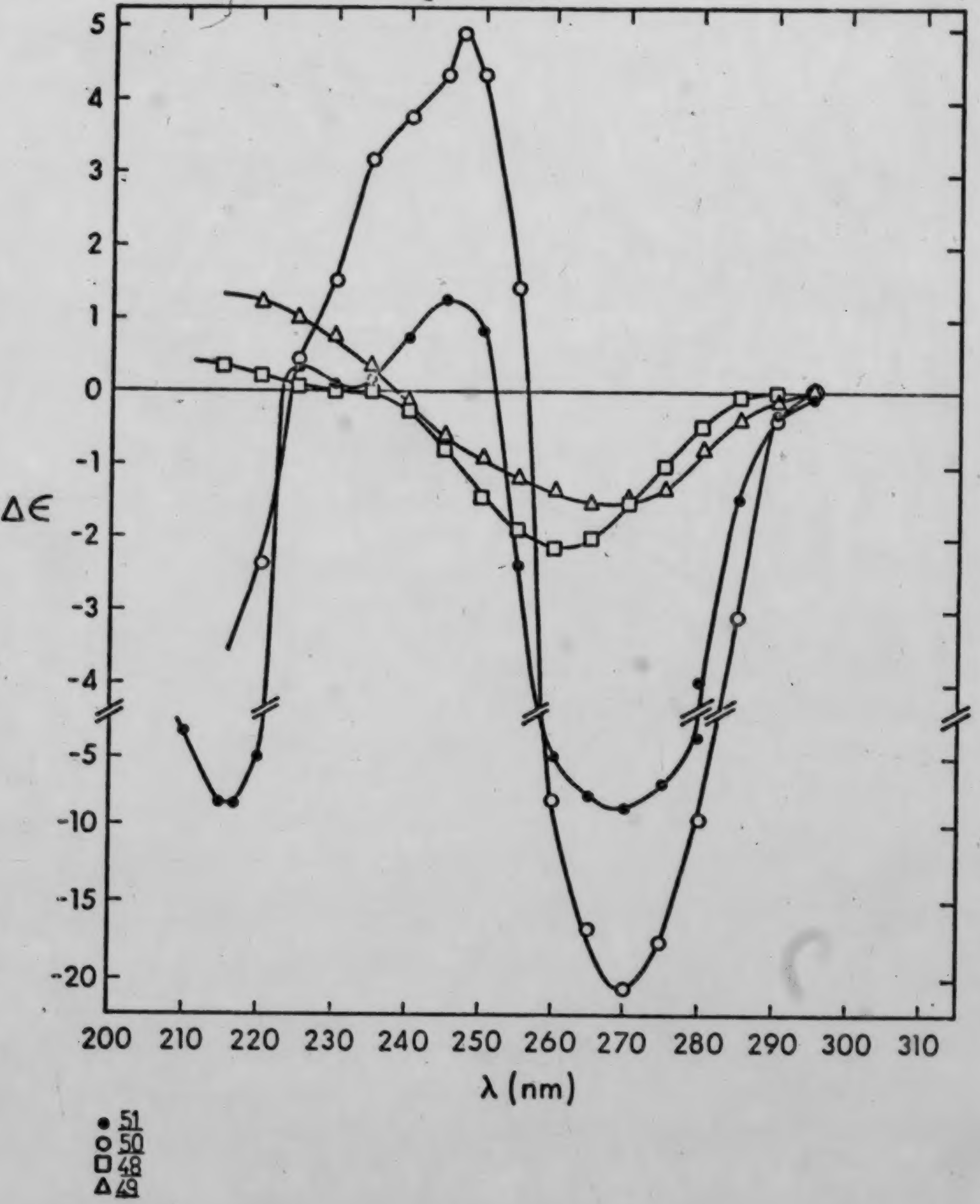
Figure 19



● 38 pH=6.2
○ 42 pH=1.1
□ 38 pH=1.1
△ 42 pH=6.2

chromophore(s). This illustrates that caution must be exercised in the interpretation of an enlarged cd effect. The cd spectra of the esters 2'-O-(48) and 3'-O-(49) p-methoxyphenylacetyl 2'(3')-O-methyl adenosines and 2'-O-(50) and 3'-O-(51) p-methoxybenzoyl 2'(3')-O-methyl adenosines further illustrates this cautionary note (see Figure 20). Neither of these isomeric pairs shows uv hypochromicity (see experimental section). This was also observed in similar compounds by Usatyi and coworkers [158] and Laporte. [159] The p-methoxyphenylacetyl derivatives exhibit only small cd effects but both p-methoxybenzoyl derivatives show large Cotton effects. The aromatic chromophore of the p-methoxyphenylacetyl derivatives is insulated by a methylene group from the optically active center (2' or 3' position), whereas with the p-methoxybenzoyl derivatives, the chromophore is attached directly via an ester linkage to the optically active centers. It is known that p-methoxybenzoyl sugars (without the nucleoside base) have large cd effects.[158] It is of interest to note that whereas 2'-O-p-methoxybenzoyladenosine has a Cotton effect at ~270 nm with $\Delta\varepsilon = -4$ and 3'-O-p-methoxybenzoyladenosine has a Cotton effect at ~270 nm with $\Delta\varepsilon = 1.5$ [158]; 50 and 51 have Cotton effects at this wavelength with $\Delta\varepsilon = -20$ and -9, respectively. This enhancement may be due to restricted rotation because of the adjacent O-methyl groups or absence of hydrogen bonding.

Figure 20


5
4
3
2
1
0
-1
-2
-3
-4
-5
-10
-15
-20
Δϵ
200
210
220
230
240
250
260
270
280
290
300
310
λ (nm)
● 51
○ 50
□ 48
△ 49

In conclusion, enhancement of the cd spectra of sugar-substituted nucleosides (compared with the underivatized parent compounds) may indicate that base-substituent overlap occurs in solution. When the enhancement is markedly pronounced in the spectrum of only one (2' specifically in the present study) of two such isomerically substituted derivatives and especially if this trend is parallel with uv hypochromicity data, the stacking interpretation is straightforward. However, enhancement can also arise by several other mechanisms. Thus, great care should be exercised in the interpretation and rationalization of such cd spectra.

ACID-CATALYZED HYDROLYSIS OF THE BASE-GLYCOSYL BOND OF SOME ADENOSINE DERIVATIVES

The following table summarizes the results of acid hydrolysis of some adenosine derivatives in 1 N HCl in dioxane : water (3:2) at 81.4°. It was noted that 2'-O-methyladenosine (32) was more stable than 3'-O-methyladenosine (17). This small enhanced stability was also noted by Reese[114] and may be due to the 2'-O-methyl group interfering in the solvation of a developing positive charge at the anomeric carbon to a greater extent than the 3'-O-methyl group. Reese[114] noted that both methylated compounds were more acid stable than adenosine. The benzyl compounds 46 and 47 displayed a further enhanced stability in acid. This stability could arise from one of the two following effects, or more likely, a combination of both. A greater interference of O-benzyl than of O-methyl with solvation at the anomeric position is likely. The slight electron withdrawing effect of the phenyl group could also destabilize the positive charge forming at C-1' in the transition state (this positive charge arises in either mechanism A or B illustrated in figure 8).

It does not appear necessary to invoke stabilization of 47 due to base sugar substituent overlap to explain these results, although this type of stabilization can not be ruled out completely. The results with the sulfonyl esters show that the most important factor stabilizing an

TABLE 1

Acid Hydrolysis Data on 2' and 3'-O Substituted Adenosines

Abbreviated Name	Compound Number	k' = 10^5, sec^{-1}*	τ' = ln2/k', min
2'-O-Methyl	32	251 (±11)	4.60
3'-O-Methyl	17	448 (±18)	2.58
2'-O-Benzyl	47	162 (±9)	7.13
3'-O-Benzyl	46	280 (±13)	4.13
2'-O-Mesyl	34	2.84 (±0.22)	407
3'-O-Mesyl	35	7.10 (±0.44)	163
2'-O-Tosyl	38	1.08 (±0.05)	1069
3'-O-Tosyl	39	3.87 (±0.24)	298
2'-O-Besyl	36	1.73 (±0.09)	668
3'-O-Besyl	37	5.37 (±0.18)	215
2'-O-Nisyl	40	0.96 (±0.14)	1203
3'-O-Nisyl	41	3.13 (±0.17)	369
2'-O-Aminosyl	42	1.56 (±0.12)	740
3'-O-Aminosyl	43	4.68 (±0.15)	247

*Average of values given in Appendix

adenosine derivative against acid-catalyzed hydrolysis is the electron withdrawing effect of the sugar substituent(s). Overlap of the base and the sugar-substituent plays a small role, if any, in the stabilization of these compounds in acid. The O-mesyl compounds which have no aromatic sugar-substituent for overlap, show remarkably enhanced stabilities. It may be argued that since the 2'-O-tosyl compound (38) displays an increased acid stability relative to the 2'-O-mesyl compound (34), this is due to the base, sugar-substituent overlap of the former compound in acid (see discussion of cd). However, it is seen that the 3'-O-tosyl derivative (39) also has an enhanced acid stability relative to the 3'-O-mesyl compound. It has been shown (see discussion of cd) that no base, sugar-substituent overlap occurs in 39. The inductive electron withdrawing effect of the p-toluyl group is greater than that of the methyl group (pK_a acetic acid = 4.75, pK_a p-toluic acid = 4.36) and this could increase the stability of both tosyl compounds compared with their respective mesyl derivatives. There could also be some enhancement in stability due to solvation effects at the anomeric position as already noted for the O-methyl parent compounds relative to the O-benzyl compounds. The besyl compounds are seen to have intermediate stabilities between the mesyl and tosyl compounds. Again the major effect can be attributed to inductive withdrawal by the sulfonyl ester group (2'-O-besyl (36) has only weak base, sugar-substituent overlap and 3'-O-besyl (37) none (see discussion on cd)). Although the benzyl group is inductively electron withdrawing (pK_a phenylacetic acid = 4.28), it doesn't stabilize compounds 36 and 37 as much as the p-toluyl group stabilizes 38 and 39. This may be due to a greater interference with solvation of the positively charged intermediate by the tosyl group compared with the besyl group in which the hydrophobic aromatic ring is removed from the anomeric position by an additional methylene group. The nisyl derivatives 40 and 41 show the greatest enhancement in stability compared with their parent O-methyl compounds, with 40 almost 500 times more stable than 17 under these conditions. Again this stability can be attributed mainly to the inductive electron withdrawing effect of the nisyl group. Some of the effect is likely due to interference with solvation at the anomeric position but the major increase from the structurally similar tosyl must be due to inductive effects. Ikehara[118] invoked an overlap interaction between the base and the phenyl group for both 2'- and 3'-O-p-nitrobenzenesulfonyladenosines to rationalize the unusual acid stability of these compounds. It has been shown that 41 does not have significant base, sugar-substituent interaction (overlap). The stability of these sulfonyl esters does not appear to be affected significantly by base sugar substituent overlap even when strongly observed by cd.

The lower acid stabilities of the 3' esters as compared to their 2' isomers is consistent with the greater distance separating the electron withdrawing substituent and the anomeric position where the positive charge is forming. It is interesting to note that the nisyl substituent offers some promise as an acid stabilizing blocking group for nucleosides. It does not migrate as carboxylic esters do [160,161] and it can easily be removed under mildly basic conditions (see experimental). The primary limitation is the moderate yields observed in the syntheses of these derivatives. It was expected that one of the most acid stable compounds would be the aminosyl derivatives. These basic substituents should be protonated and thereby be among the strongest electron withdrawing substituents. It was found, however, that the aminosyl group was hydrolytically removed under the conditions of these hydrolyses. The sulfanilic acid released was isolated and identified by comparison with an authentic sample by chromatography and uv spectra in acid and base. The aminosyl group would not be expected to cleave from the sugar products of hydrolysis at a greater rate than from the original nucleoside. Also, sulfanilic acid appeared immediately in the hydrolysis. The absence of a "lag time" suggested a slow continual hydrolysis of the sulfanilate substituent. This would give:

base-sugar-ester $\xrightarrow{k_1}$ base + sugar-ester

$\xrightarrow{k_2}$ base-sugar + ester $\xrightarrow{\text{fast}}$ base + products.

Whereas the hydrolyses have been assumed to go *via* direct base-sugar cleavage, this new path of hydrolysis may now be operative forming the less stable parent nucleoside which is rapidly hydrolyzed (Table I) to the base and sugar products. The rate of disappearance of the aminosyl derivatives could then be dependent on two phenomenological first order rate constants, k_1 and k_2, whose sum would correspond to the measured pseudo first order rate constant, (Rate = k_1[substrate] + k_2[substrate] = k'[substrate], where $k' = k_1 + k_2$).

This raises the possibility that all of the hydrolyses go in part by prior ester cleavage although the previously discussed sulfonyl ester derivatives qualitatively follow an electronegative trend. The initial goal of this hydrolysis study was to investigate the role of base, sugar substituent overlap in the acid stability of these derivatives. Elucidation of a consistent and detailed mechanism for their acid catalyzed hydrolysis will require further research. As predicted the 2'-*O*-aminosyl derivative (**42**) displays a pronounced acid stability relative to its 3'-isomer (**43**) although there is no evidence for any base-sugar substituent overlap in acid

Figure 21

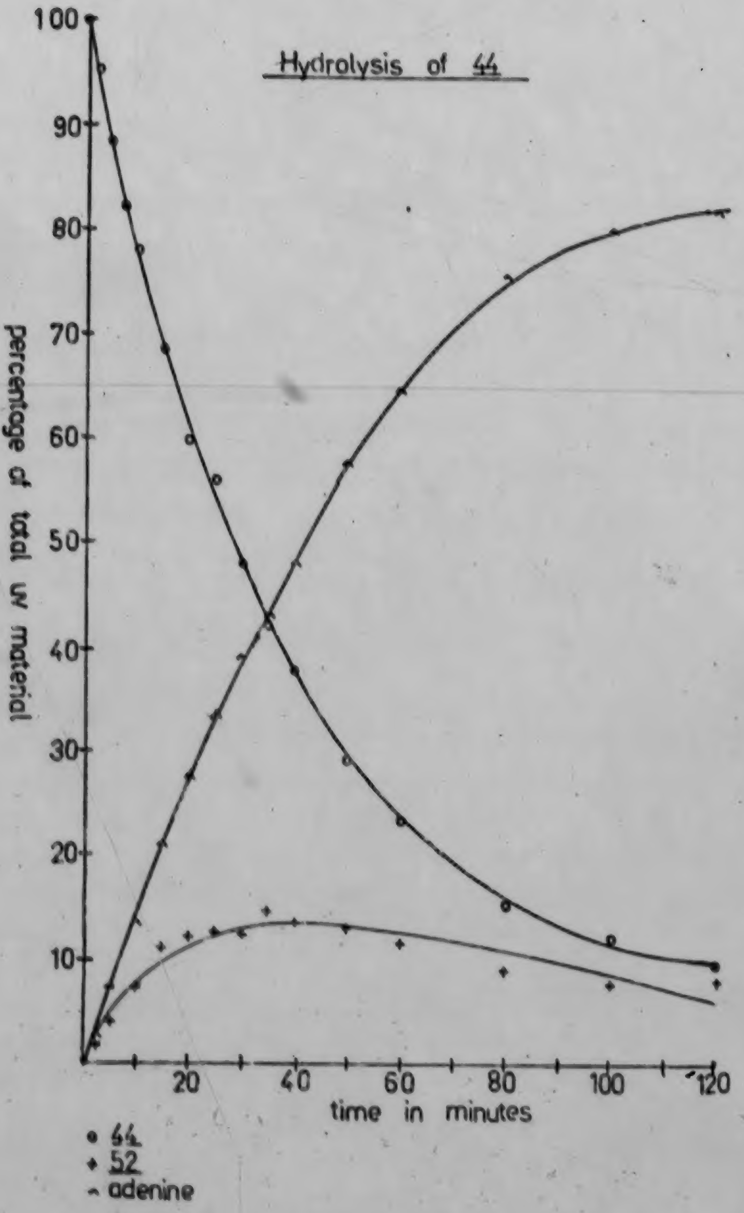
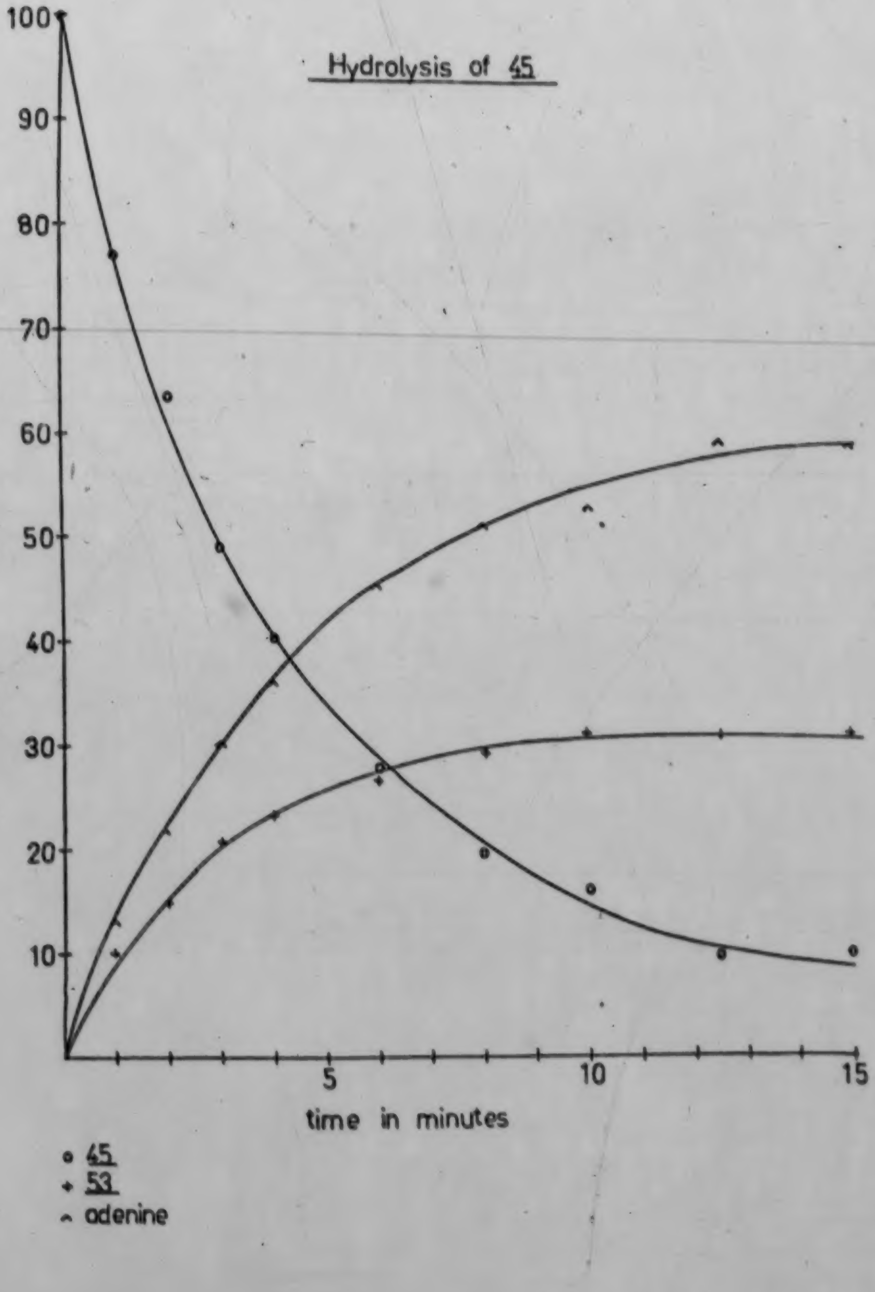

Hydrolysis of 44
percentage of total uv material
100
90
80
70
60
50
40
30
20
10
20
40
60
80
100
120
time in minutes
○ 44
+ 52
^ adenine

Figure 22


Hydrolysis of 45
100
90
80
70
60
50
40
30
20
10
percentage of total uv material
5
10
15
time in minutes
○ 45
+ 53
^ adenine

solution (see uv discussion).

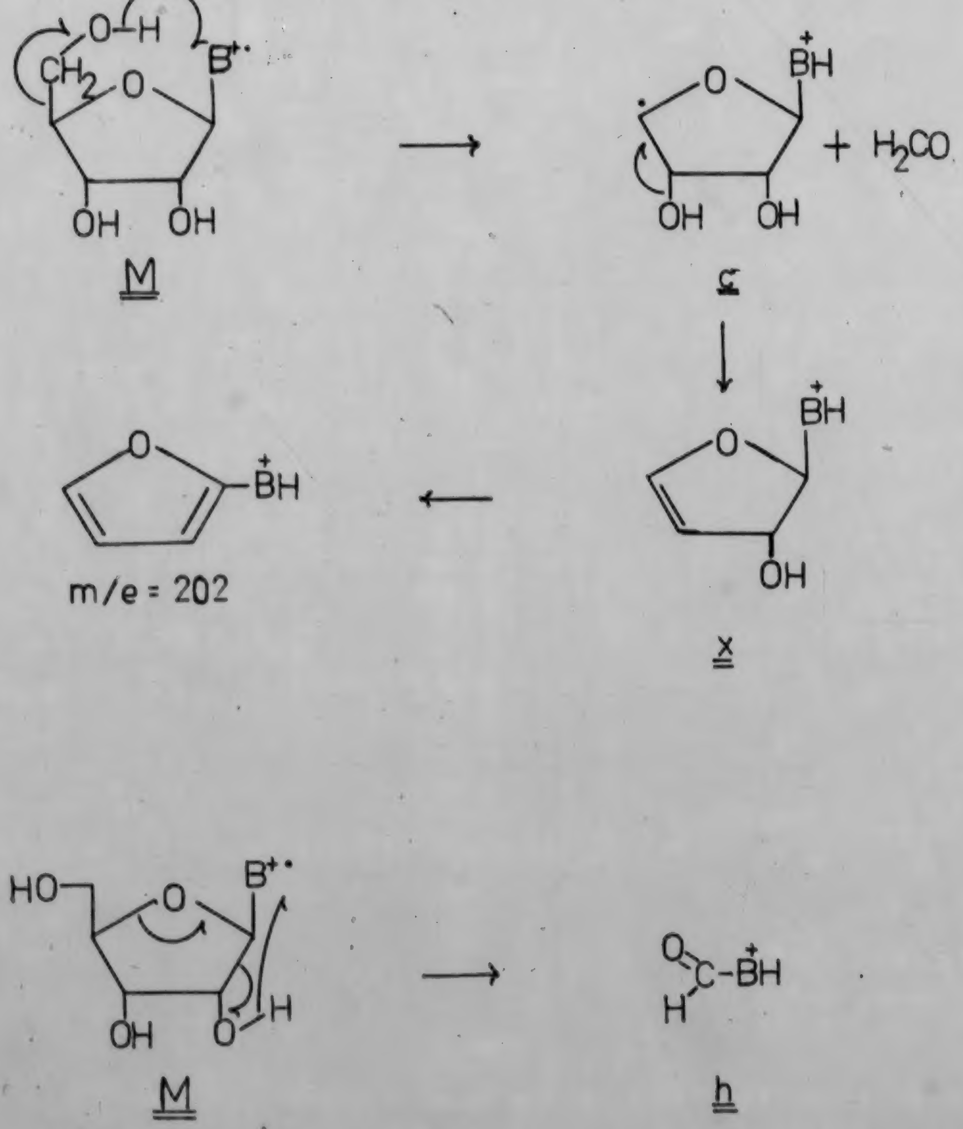
Hydrolysis of the triflate esters 44 and 45 is even more complex. In both cases a long lived biproduct was formed (see Figures 21 and 22). In the case of 44, a 2'-chloro-2'-deoxy-3'-O-methyl nucleoside product (52) was formed and the corresponding 3'-chloro-2'-O-methyl product (53) was formed from 45 (both identified by ms). In each case the chloro biproduct had a longer half-life than the triflate starting material giving rise to an appreciable build up of the biproducts. Since the triflate esters contain the strongest electron withdrawing substituent in the table, one would expect them to be very stable against hydrolysis. There was no "lag time" in the appearance of adenine as would have been expected if the hydrolyses went solely via the chloro biproducts so it would appear that some transient acid sensitive intermediate, possibly an unsaturated elimination product, was involved in each case. It is not surprising that the triflates exhibit instability in the hot, acidic solution since they also displayed a tendency to decompose during their syntheses (see the experimental section).

In conclusion, it is noted that the dramatic increase in acid stability exhibited by certain of the adenosine sulfonyl ester derivatives prepared in this thesis is due primarily to the stabilizing effect of electron withdrawint substituents on the sugar.

MASS SPECTRA

McCloskey and coworkers [162] have investigated mass spectrometry of a wide variety of adenosine derivatives. They have determined the principle fragmentation pathways for structurally significant ions and have postulated decomposition mechanisms based on metastable transitions, deuterium and substituent labels and high-resolution peak identification. The most significant ions in adenosine derivatives consist of the purine base (adenine) plus various portions of the sugar skeleton, reflecting stability of the aromatic base toward decomposition. The sugar fragment (ion s) plays a minor role in the fragmentation of most adenosine derivatives. Ions that occur in the high mass region of the spectrum are usually very easily detected, even at low intensity, since they are not obscured by other fragments. The molecular ion M^+ and often minor ions corresponding to M-OH or in the case of sugar methylation $M-OCH_3$ occur in the high mass region. $(M-OH, M-OCH_3)$ do not involve specific hydroxyl or methylated hydroxyl groups and therefore are of no structurally diagnostic value. Elimination of OH_2C-5' as formaldehyde (30 mass units), which occurs with accompanying proton transfer from the 5'-hydroxyl group to the base, leads to an important structural indicator, ion c, corresponding to M-30 (see Figure 23). Ion c occurs in compounds that

Figure 23


M
c
+ H_2CO
x
m/e = 202
M
h

possess the free 5'-hydroxymethoxy group. Often molecules that have the 5' position blocked or substituted will exhibit a fragmentation that involves loss of the entire 5' group without concomitant transfer of a proton to the base. Ion c is also thought to lead, by the fragmentation pathway shown in Figure 23, to ion x and an ubiquitous fragment ion at m/e 202. These ions are more intense in compounds with a labile C-3' or C-2' substituent. Ion x is useful in determining C-2' substitution but is complicated by the fact that many adenosine derivatives also show an ion corresponding to loss of the 2' substituent from ion c.

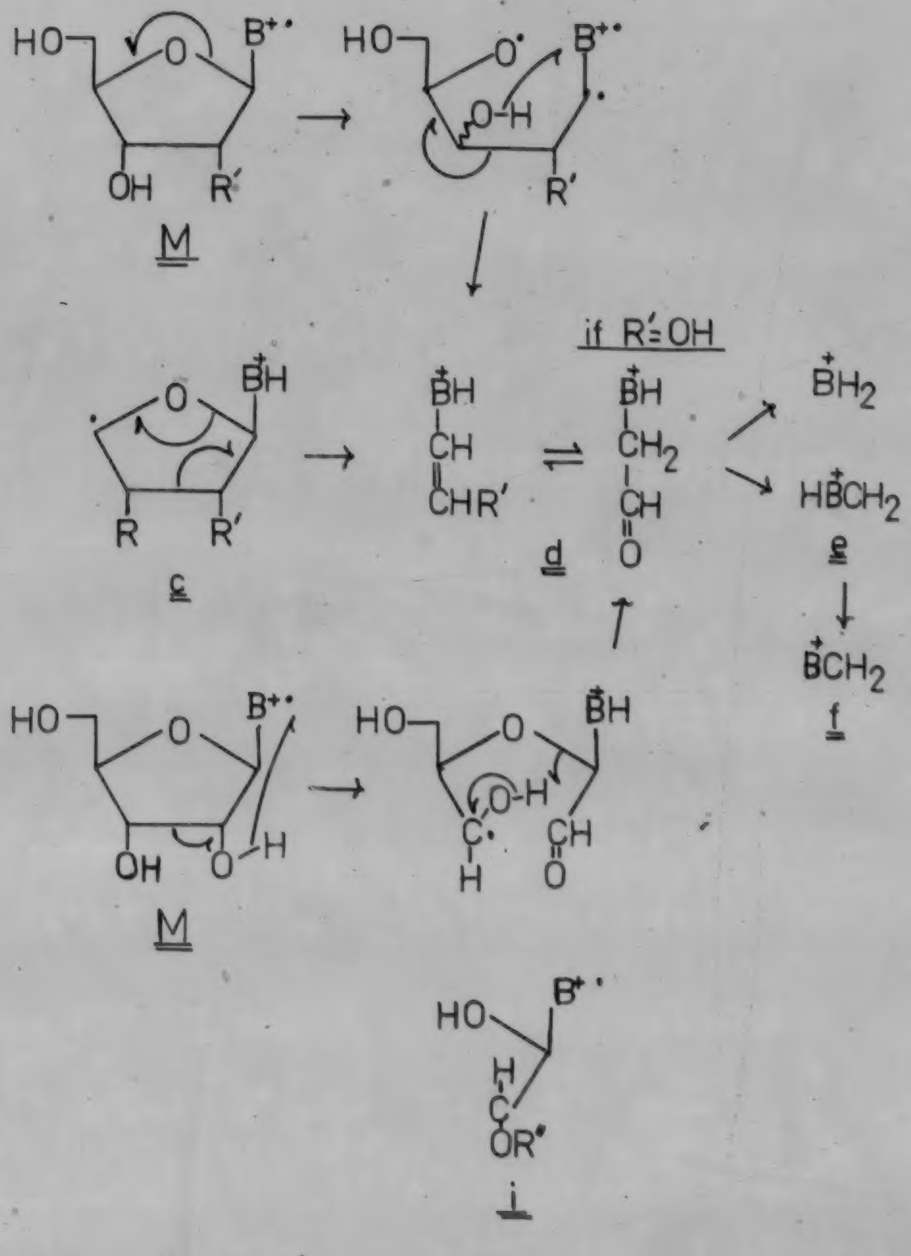
A more useful ion for determination of C-2' substitution is ion d which occurs at the mass of the base (B) plus 44 mass units in the case of adenosine. This prominent ion shifts according to the mass of the C-2' substituent and was shown by McCloskey [162] to involve more than one mechanism. Three mechanisms proposed by McCloskey are outlined in Figure 24. Also shown are possible routes of formation of ions e (B + 15 mass units), f (B + 14 mass units) and B + 2H, a major ion which will be discussed below. These ions are also formed via more than one mechanism.

Ion h (B + 30 mass units for adenosine) reflects structural changes at C-1'. McCloskey [162] proposed the mechanism given in Figure 23. This mechanism requires a 2'-hydroxyl hydrogen but again ion h must be formed by other routes since there are numerous examples (see experimental section) where a significant ion h is formed without a labile hydrogen at the 2' position.

Two other common peaks which occur in nucleoside mass spectra are ion i and ion j. Ion i, which in adenosine consists of the base plus 60 mass units of the sugar, was proposed by McCloskey to contain C-1' and C-2' plus their attached oxygens (see Figure 24). This ion could result from homolysis of the 2' to 3' bond followed by transfer of a C-5' or C-3' hydrogen to the ring oxygen. This ion is of some use in determining C-2' substitution. Ion j (m/e 190) which consists of the base plus 56 mass units of the sugar was found by McCloskey [162] to contain the base, C-1', C-2', C-3' and one heteroatom of the sugar. With 2' and 3'-O-methyl compounds, a related ion y (m/e 204) consisting of the base, C-1', C-2', C-3' and OCH_3 could be seen. Also with the sugar methylated nucleosides a peak at m/e 173 ($y-OCH_3$) frequently occurred.

Perhaps the most characteristic ions in nucleoside mass spectra (often the most intense) are those representing the protonated base (B + H and B + 2H). These ions vary in mass with substitution of the base and are useful in identifying this substitution. Another peak that

Figure 24


HO
B+•
O
OH
R′
M
O•
O-H
c
BH
R
CH
CHR′
d
if R′=OH
CH2
CH
O
BH2
HBCH2
e
BCH2
f
H
C•
OR″
i

occurs in certain cases in 2'-O-methylated compounds is ion v (m/e 177) which represents loss of the methyl group from ion d. Ion w which corresponds to the sugar (s) less one hydrogen atom also appears as a fragment in some compounds.

Substituents, of course, also have characteristic fragmentations. The trityl group, for instance, gives rise to numerous peaks. Besides the very large fragments at m/e 243 ($C\phi_3$), there are other smaller peaks at m/e 241, 239, 228, 215, 183 and 165 among others. To illustrate the usefulness of mass spectral data in the identification of nucleoside analogues, two examples are discussed below.

The first example is the product (54) of the reaction of xylosyladenine with thionyl chloride in HMPA. Accurate mass measurements showed that the product was monochlorinated (M peak at m/e 285). Ions B + H and B + 2H at m/e 135 and 136, respectively, showed that the compound was not base substituted. Ion d at m/e 178 and i at m/e 194 showed that the compound was not chlorinated at C-2'. The absence of ion c and the presence of an ion at m/e 236 corresponding to $M-CH_2Cl$ indicated that the chlorine substitution had occurred at the 5' position.

The second example involves the two byproducts of hydrolysis of 44 and 45, in which only a small amount of material was available. Both compounds detected appeared to be monochlorinated derivatives of O-methylated adenosines (M, m/e 299). Both exhibited the absence of base substitution (B + H, B + 2H at m/e 135 and 136, respectively) and were not 5' substituted nucleosides (ion c at m/e 269). The product isolated from the hydrolysis of 44 had ion d at m/e 196 while the corresponding product from hydrolysis of 45 had ion d at m/e 192. This suggested that the former was chlorinated at C-2' and the latter at C-3' (with the other position O-methylated). The very intense ion x at m/e 234 for the latter compound is also indicative of a good leaving group at C-3'. These assignments were correlated with the isotope ratios of chlorine (^{35}Cl: ^{37}Cl, 100 : 32.6) for halogen-containing ions.

Thus, it can be seen that mass spectrometry is a very valuable tool for the identification of nucleosides, especially when only very small amounts of nucleosidic material are available.

NUCLEAR MAGNETIC RESONANCE SPECTRA

Proton nuclear magnetic resonance spectroscopy (^{1}H nmr) represents a powerful tool in the identification of nucleoside derivatives. All spectral samples were dissolved in either $(CD_3)_2SO$ (DMSO-$\underline{d}_6$) or $CDCl_3$ and a comparison of the resulting nmr with one run after addition of D_2O, determined the exchangeable hydrogens.

Generally theprotons appearing furthest downfield are H^8 and H^2 of the base. These usually appear as singlets at ~8.30 and 8.10. The next non-exchangeable hydrogen observed was the anomeric proton $H^{1'}$ at δ ~6.0. The anomeric proton was coupled to only the C-2' protons and was not overlapped by any other non-exchangeable protons. It also appeared as the furthest downfield sugar proton for all compounds in this thesis. A first order analysis of the coupling constants (with decoupling in some cases) was used to identify the remaining sugar protons. The multiplicity of the anomeric proton identified the sugar C-2' as either deoxy (X doublet of doublets of an ABX system) or monosubstituted (AX doublet). Using this coupling constant, $H^{2'}$ (and $H^{2''}$ if present) was identified. After deuteration of exchangeable protons, the remaining coupling with $H^{2'}$ was the C-3' proton(s). Thus, from the multiplicity of $H^{2'}$ (and $H^{2''}$ if present) the C-3' proton(s) were identified as substituted or as deoxy. This procedure was extended to identify $H^{4'}$, $H^{5'}$ and $H^{5''}$.

The degree of asymmetry of the splitting patterns was also helpful in identifying or confirming the identity of protons. For example, the upfield peak of the anomeric doublet increased in intensity (with respect to the downfield peak of the doublet) inversely proportional to the chemical shift difference between the anomeric and the $H^{2'}$ protons. That is, the smaller the chemical shift difference, the larger the relative magnitude of the upfield peak. This "skewing" of the peaks was of great help in making many assignments. Again, where possible, the assignment of peaks was verified by spin-spin decoupling experiments.

The chemical shifts of the various sugar protons were very useful in assigning the position of functionalization (see synthesis of 2'-deoxyadenosine compounds). Electronegative groups such as acetyl, benzoyl and sulfonyl groups markedly shift the sugar proton attached to the substituted carbon downfield. Neighboring protons were shifted downfield to a lesser degree. Rules formulated by Reese and coworkers were applied and were useful in making assignments with compounds in which either the C-2' hydroxy or the C-3' hydroxy (but not both) were substituted. These rules state that for a pair of 2' and 3' isomers, the $H^{1'}$ resonance is at lower field and $J_{1'-2'}$ is smaller for the 2' isomer.

Since a large number of isomeric *O*-methyl compounds are described in this thesis, it is interesting to compare their nmr spectra (see Table I). It can be seen that the position of the electronegative sulfonyl or acyl group can easily be determined by the chemical shift. Thus 2'-*O*-substituted derivatives (sulfonyl or acyl) have shifts to lower field for $H^{2'}$ and 3'-*O*-substituted derivatives have shifts to lower field for $H^{3'}$. Also, if the electronegative substituent is considered the determining group, all compounds follow the rule of Reese [163] stated above except for the *p*-toluenesulfonyl derivatives. In the case of the tosyl compounds $H^{1'}$ of the 2' isomer is at lower field but also has a larger $\underline{J}_{1'-2'}$ value. Since this coupling reflects the conformation of the ribose ring, it seems that in this case the sugar conformation has changed. This may be due to base, sugar-substituent overlap. (See discussion of cd). Also in the case of the 2'-*O*-tosyl, nisyl and aminosyl derivatives, there is a marked upfield shift of H^2 and H^8 compared with the 3'-*O*-substituted derivatives. Although it is known that base stacking does not occur in DMSO-$\underline{d}_6$ with dinucleotides [164], the nmr shifts of H^2 and H^8 in the case of the above mentioned sulfonyl derivatives may signify base substituent stacking. The fact that the sulfonyl compounds exhibit this stacking in DMSO-$\underline{d}_6$ while dinucleotides do not may result from the smaller distances involved

TABLE 2

^{1}H Nmr Data for Sugar Substituted Adenosine Derivatives

Abbreviated Name	Compound Number	Proton nmr Shift in units							Substituent Proton Shifts			
		$H^{5'}$	$H^{4'}$	$H^{3'}$	$H^{2'}$	$H^{1'}$	H^2	H^8	alkyl	aromatic protons		$\underline{J}_{1'-2'}$ in Δδ
2'-O-Mesyl	34	3.71	4.13	4.18	5.72	6.15	8.16	8.38	3.43			4.8
3'-O-Mesyl	35	3.71	4.37	5.41	4.79	6.03	8.16	8.39	3.32			7.1
2'-O-Besyl	36	3.68	~4.19	~4.19	5.72	6.21	8.17	8.36	4.76	7.25		5.3
3'-O-Besyl	37	3.63	4.26	5.37	4.76	6.02	8.17	8.41	4.83	7.42		7.3
2'-O-Tosyl	38	3.60	~4.11	~4.11	5.56	6.06	7.97	8.14	2.16	7.02	7.37	7.4
3'-O-Tosyl	39	3.51	4.10	5.32	4.70	6.01	8.16	8.37	2.43	7.52	7.92	7.2
2'-O-Nisyl	40	3.62	~4.14	~4.14	5.73	6.09	7.97	8.18		7.89	8.13	6.2
3'-O-Nisyl	41	3.54	4.13	5.49	4.76	6.01	8.15	8.36		8.29	8.50	7.0
2'-O-Aminosyl	42	3.60	4.12	3.95	5.54	6.08	8.09	8.20		6.45	7.25	7.0
3'-O-Aminosyl	43	3.48	4.06	5.12	4.62	5.98	8.14	8.36		6.70	7.60	7.6
2'-O-Triflate	44	3.72	4.20	4.39	6.11	6.41	8.18	8.40				4.5
3'-O-Triflate	45	3.68	4.42	5.84	5.06	6.07	8.24	8.39				7.8
2'-O-p-Methoxyphenylacetyl	48	3.63	~4.17	~4.17	5.78	6.17	8.15	8.36	3.63	6.79	7.10	5.6
3'-O-p-Methoxyphenylacetyl	49	3.66	4.16	5.49	4.70	6.01	8.16	8.41	3.73	6.89	7.24	7.3
2'-O-p-Methoxybenzoyl	50	3.73	4.24	4.36	5.97	6.34	8.20	8.46		7.07	7.95	5.5
3'-O-p-Methoxybenzoyl	51	3.76	4.33	5.71	4.81	6.16	8.21	8.48		7.11	8.05	6.8

in the case of the sulfonyl compounds and thus stronger interactions. The same effect is observed on the protons of the aromatic substituent. Although the anisotropic effect of the base causes some shielding of the 2' substituent (see mesyls, besyls, p-methoxyphenylacetyls and p-methoxybenzoyls) there is an increased shielding effect for the 2'-O-tosyl, nisyl and aminosyl. This again suggests overlap of the base and the 2' aromatic substituent.

EXPERIMENTAL

A. GENERAL PROCEDURES

Melting points were determined on a Reichert microstage apparatus and are uncorrected. Nmr spectra were recorded on Varian HA-100 and Brücker 90 spectrometers with TMS as reference. Uv spectra were recorded on Cary 15 or Pye Unican SP 1700 spectrophotometers. Optical rotations were determined with a Perkin Elmer Model 141 polarimeter using a 10 cm, 1 ml microcell. Cd spectra were recorded on a Jasco Optical Rotatory Dispersion Recorder with an SS-20-2 modification using a 1mm cell. MeOH used for uv and cd spectra was spectral grade. Dimethylformamide (DMF), used for optical rotations, was distilled from calcium hydride under reduced pressure at <40° and stored over Linde 4A molecular sieves.

Mass spectra (ms) were determined by the mass spectrometry laboratory on AEI MS-2 or MS-9 indstruments at 70 eV using a direct prove. Mass spectra are quoted as per cent relative intensity of the most intense ion at m/e > 134. Elemental analyses were determined by the microanalytical laboratory or by Schwarzköpf Microanalytical Laboratory, Woodside, New York. Water of hydration was verified in the nmr spectrum. When DMSO-$\underline{d}_6$ was used as solvent a "blank" was recorded and the relative intensities of the H_2O and DMSO-$\underline{d}_6$ peaks was calculated. The amount of H_2O in the sample was then determined by subtracting this blank value from the integrated H_2O peak.

TLC was performed on Eastman Chromatogram sheets (silica gel No. 13181, Indicator No. 6060) in the solvent system indicated. Developed chromatograms were evaluated under 2537 Å light. Preparative scale TLC was performed on ~1 mm thick Merck silica gel GF 254 (20 x 20 cm) plates. Evaporations were carried out using a Büchler rotating evaporator with aspirator or oil pump vacuum at <40°. Co-evaporations were effected by adding the specified solvent and re-evaporating. Hydrogenations were accomplished using a Paar shaking apparatus at room temperature at the specified hydrogen pressure with Matheson, Coleman and Bell 5% palladium on carbon as catalyst. Tri-n-butyltin hydride solution was prepared by the Holý modification [59] of the original procedure.[165] Column chromatography was performed using J. T. Baker No. 3405 silica gel (except as noted, where Herst: Gebr. Kieselgel was used) or Woelm II alumina. Electrophoresis was performed on Whatman number 1 paper using a Savant flatplate apparatus (HV-3000 A). Descending chromatography was effected using Whatman number 1 paper. The procedure used to make the "borate" solvent system [168] was: 1 M ammonium acetate containing 0.01 M ethylenediamine-tetraacetic acid di-sodium salt was adjusted to pH 9 with ammonium hydroxide and saturated with sodium tetraborate. This solution (60 ml) and 90% EtOH (140 ml) were mixed and allowed to stand for 1 h. The precipitate which formed during this time was filtered before use. Solvent system E (SSE) is the separated upper phase of EtOAc:n-propanol:H_2O 4 : 1 : 2 (by volume). Diffusion crystallization, which has been modified in this laboratory[166,167] was effected by allowing a concentrated solution of the nucleoside in the first mentioned (dissolving) solvent to stand in a desiccator containing a large volume of the second (diffusing) solvent (in which the material is insoluble) at room temperature and the resulting crystals were collected by filtration.

Pyridine was refluxed over and distilled from calcium hydride before use. Toluene was dried using sodium wire. p-Dioxane was purified as described by Vögel.[169] EtOAc was distilled from P_2O_5 and stored over Linde 4A molecular sieves (dried at 200°). Other solvents used were of reagent purity and were distilled before use. Pivalyl chloride, methanesulfonyl chloride and $SOCl_2$ were distilled before use. All reagent solids were recrystallized before use. The 3'-O-methyladenosine and 2'-O-methyladenosine were prepared as described.[122] Adenosine was purchased from Raylo Chemicals Ltd., Edmonton, Alberta. Dr. Yves Fouron kindly provided the 9-(β-D-xylofuranosyl)adenine.

B. HYDROLYSES

Acid catalyzed hydrolyses were effected by dissolving a sample of nucleoside in a solution of 1,4-dioxane: 2.5 N HCl (3:2) to give a final substrate concentration of 2.5×10^{-5} mole/ml. Samples (20 µl) were injected into small glass capillaries (sealed at one end) and the other end of the capillary was then sealed. The entire set of capillaries was submerged at "t ≈ 0" into a 10 gallon oil bath at 81.40° ± 0.05° (checked with a Hewlett Packard 2801A Quartz Thermometer). At recorded intervals these capillaries were removed and rapidly submerged in a dry ice, acetone bath.

ANALYSIS OF HYDROLYSES

Method A

The capillaries were opened and the contents applied to a strip of Whatman #1 paper which was developed in isopropanol : H_2O : concentrated aqueous NH_3 (7 : 2 : 1) or else EtOH : H_2O (7 : 3). The appropriate spots were cut out and eluted with 0.1 N HCl (4 ml or 6 ml depending on the extinction coefficient). The resulting uv absorption maximum of the solution was recorded and a blank value (obtained from an identical size piece of chromatography paper treated in an identical manner) was subtracted. The quantities of substrate and product were then calculated.

Method B

The capillaries were opened and 0.2 µl aliquots were analyzed using high performance liquid chromatography (HPLC; Tracor 5000 chromatographic pump), employing a silica gel column (1 m x 2 mm I.D.; Reeve Angel PPC41 HS Pellosil) with isopropanol : H_2O : concentrated aqueous NH_3 : CH_2Cl_2 (350 : 1 : 2 : 647) as solvent. The areas under the respective peaks monitored at 254 nm were determined using a Mini Lab Integrator (Model CSI 38 Digital Integrator; Columbia Scientific Industries). A similar analysis of the data was carried out as described above.

The equilibrium time (~12 seconds) for 20 µl of the hydrolysis solvent [p-dioxane : 2.5 N HCl (3 : 2)] to reach 81.4° was determined using a thermocouple (Biddle Grey Instruments TC Potentiometer) in an open capillary tube under the experimental conditions. Therefore, a "t ≈ 0" time of at least 30 seconds after immersion of the samples in the bath was used for the adenosine ether derivatives. Each subsequent determination was corrected for the amount of hydrolysis occurring up to that "t ≈ 0" time. This correction was negligible with the sulfonyl ester derivatives with longer half lives.

Derivation of the rate equation:

Two possible explanations for the phenomenological first order rate constants (k') observed are:

A All productive hydrolysis events proceed via a minor protonated form in low equilibrium concentration.

$$AR + H^{+} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ARH^{+}$$

$$AR + H^{+} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} BRH^{+}$$

$$BRH^{+} \xrightarrow{k_d} \text{Products}$$

Where AR = adenosine derivative, ARH^{+} = major protonated species, BRH^{+} = minor protonated species which undergoes hydrolysis, k_1 and k_{-1} = rate constants of protonation and deprotonation of major protonated species, k_2 and k_{-2} = rate constants of protonation and deprotonation of minor protonated species, k_d = rate constant of hydrolysis of minor species.

$$[ARH^{+}] = \frac{k_1}{k_{-1}} [AR][H^{+}] \text{ and } [AR][H^{+}] = \frac{k_{-2}}{k_2} [BRH^{+}]$$

$$\therefore \quad 1. \qquad [ARH^{+}] = \frac{k_1 k_{-2}}{k_{-1} k_2} [BRH^{+}]$$

$$2. \qquad \frac{-d[AR]}{dt} = k_1[H^{+}][AR] - k_{-1}[ARH^{+}] + k_2[H^{+}][AR] - k_{-2}[BRH^{+}]$$

Assuming a steady state for $[BRH^{+}]$

$$\frac{-d[BRH^{+}]}{dt} = k_{-2}[BRH^{+}] + k_d[BRH^{+}] - k_2[H^{+}][AR] \simeq 0$$

$$\therefore \quad 3. \qquad [BRH^{+}] = \frac{k_2}{k_{-2} + k_d} [H^{+}][AR]$$

Combining 1,2, and 3.

$$\frac{-d[AR]}{dt} = \frac{k_d(k_1 + k_2)}{k_{-2} + k_d}[H^+][AR]$$

or

$$\frac{-d[AR]}{dt} = k'[AR] \quad \text{where } k' = \frac{k_d(k_1 + k_2)}{k_{-2} + k_d}[H^+]$$

B Hydrolysis proceeds in accordance with the more complex equation derived by Zoltewicz [95] which included mono and diprotonation equilibria and rate determining A-1 hydrolysis of those cationic species.

rate = $k\psi[S]_t$ or rate = $k'[S]_t$ where $k' = k\psi$ and ψ is the derived expression [95] containing mono and diprotonation equilibrium constants, hydrolysis constants and hydrogen ion concentration. Under the conditions in this thesis $[S]_t = [AR]$ (measured after neutralization). At constant hydrogen ion concentration [which is closely approximated in the 1 N acid solution with ~0.02 N concentrations of nucleosides which give product adenine of comparable basicity (pK_a)[170]], both treatments reduce to the same phenomenological pseudo first order rate constant k'.

$$k'[AR] = \frac{-d[AR]}{dt}$$

$$k'\int_0^t dt = -\int_{C_0}^{C} \frac{d[AR]}{[AR]}$$

$$k't = \ln[AR]\Big|_{C}^{C_0}$$

$$k' = \frac{1}{t}\ln\left(\frac{[AR] + [A]}{[AR]}\right) \quad \text{at } t > t_0$$

For Method A: $[A] = A_{max}/\varepsilon_{max}$ of adenine

$[AR] = A_{max}/\varepsilon_{max}$ of nucleoside substrate

For Method B: [A] set equal to HPLC integration of adenine peak

[AR] = integrated nucleoside HPLC peak x normalization factor

$$\text{normalization factor} = \frac{\varepsilon_{254\ nm} \text{ of adenine}}{\varepsilon_{254\ nm} \text{ of nucleoside}}$$

to correct to the same molecular ratio at the detector wavelength.

Comparisons involving the different derivatives are assumed to be valid since the stock acid solution and conditions of hydrolysis were identical and equilibrium acidity constants for sugar-substituted adenosine derivatives [which hydrolyze to the identical product base (adenine)] are closely comparable.[170]

Table I contains the values for k' and Table 3 the $\varepsilon_{254\ nm}$ values used for the normalization factor. The Appendix contains the experimental hydrolysis data, method of data treatment, and paper chromatographic R_f values.

TABLE 4

Extinction Coefficients at 254 nm of Sugar Substituted Adenosine Derivatives

Abbreviated Name	Compound Number	uv εmax at 254 nm in HPLC solvent
Adenine	1	10,800
2'-O-Methyl	32	13,400
3'-O-Methyl	17	13,600
2'-O-Benzyl	47	12,100
3'-O-Benzyl	46	13,600
2'-O-Mesyl	34	12,000
3'-O-Mesyl	35	12,000
2'-O-Tosyl	38	10,300
3'-O-Tosyl	39	12,000
2'-O-Besyl	36	12,200
3'-O-Besyl	37	12,700

C. SYNTHESIS

9-(3-*O*-Methyl-5-*O*-triphenylmethyl-β-*D*-ribofuranosyl)adenine (18)

To a stirred solution of 6.36 g (0.0227 mole) of 9-(3-*O*-methyl-β-*D*-ribofuranosyl)adenine (dried by repeated evaporation of dry pyridine) in ~100 ml of dry pyridine was added 5.7 g (0.0204 mole) of triphenylmethyl chloride. The solution was heated, with exclusion of moisture, for 2 h at 100° in an oil bath and then cooled. Pyridine was evaporated to give a volume of ~50 ml and this solution was poured into 150 ml of saturated, cold, aqueous $NaHCO_3$ solution. The resulting mixture was extracted with 250 ml of $CHCl_3$. The $CHCl_3$ extract was washed with 75 ml of saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and evaporated to dryness. Residual pyridine was removed by coevaporation of dry toluene (2 x 100 ml). The resulting yellow amorphous solid was taken up in a small amount of $CHCl_3$ and applied to a silica gel column (5.5 x 40 cm, 300 g) packed in $CHCl_3$. The column was washed with $CHCl_3$ (4.2 l) to remove all of the triphenylmethanol and product was eluted with MeOH : $CHCl_3$ (1 : 24). The first 1.5 l of eluate was discarded and the following 1.8 l was evaporated. Crystallization of the residue from acetone gave 5.92 g (50%) of 18. The original aqueous $NaHCO_3$ layer and extracts were combined and continuously extracted with CH_2Cl_2 to give 1.51 g of starting material, giving a yield of 65.5% for 18, based upon recovered starting material. Pure 18 had mp 120-122°; uv (MeOH)max 259 nm (ε 16,300), min 240 nm (ε 9,000); nmr (DMSO-$\underline{d}_6$) δ 3.26 (m, 2, $H^{5'}$, $H^{5''}$), 3.37 (s, 3, OCH_3), 4.08 (m, 2, $H^{3'}$, $H^{4'}$), 4.90 (m, $\underline{J}_{2'-1'}$ = 4.8 Hz, $\underline{J}_{2'-OH}$ = 6.0 Hz, $\underline{J}_{2'-3'}$ = 4.5 Hz, 1, $H^{2'}$), 5.55 (d, $\underline{J}_{2'-OH}$ = 6.0 Hz, 1,2'-OH), 5.92 (d, $\underline{J}_{1'-2'}$ = 4.8 Hz, 1, $H1'$) 7.31 (m, 17, C $_3$, 6-NH_2), 8.12, 8.27 (s,s; 1,1; H^2, H^8); ms (200°) m/e 523 (0.1, M), 493 (0.1 M-30), 388 (0.08, w), 280 (100, M-$C\phi_3$), 264 (3, c), 243 (56, $C\phi_3$), 241 (5.5), 239 (4), 228 (6), 215 (4), 202 (2.5), 190 (1,j), 183 (1.5), 165 (66, $C\phi_3$ -ϕ), 164 (21, h), 152 (2), 148 (7.5, f), 136 (55, B + 2H), 135 (16, B ± H).

Anal. Calcd for $C_{30}H_{29}N_5O_4$: C, 68.82; H, 5.58; N, 13.38. Found: C, 68.79; H, 5.78; N, 13.61.

9-(2-O-Acetyl-3-O-methyl-β-D-ribofuranosyl)adenine (20)

To a stirred solution of 8.1 g (0.0155 mole) of 18 (dried by repeated evaporation of dry pyridine) in ~80 ml of dry pyridine, cooled in an ice bath, was added 1.72 ml (0.0155 mole) of acetic anhydride dropwise with exclusion of moisture. This solution was stirred for 18 h at 5° and a further 0.15 ml (0.014 mole) of acetic anhydride was added. After 2 h stirring at 5°, the reaction was allowed to warm to 19° and 0.5 ml (0.0045 mole) of acetic anhydride was added. After 1.5 h at this temperature, the reaction mixture was poured, with stirring, into 200 ml of ice water and extracted with $CHCl_3$ (3 x 100 ml). The combined organic phase was washed with cold 10% NaHCO$_3$ solution (100 ml) and H$_2$O (100 ml) and then evaporated to dryness. Residual pyridine was removed by coevaporation with dry toluene (2 x 50 ml) leaving 8.45 g of 9-(2-*O*-acetyl-3-*O*-methyl-5-*O*-triphenylmethyl-β-*D*-ribofuranosyl)adenine (*19*) as a white amorphous solid; nmr (CDCl$_3$) δ 2.34 (s, 3, OCOCH$_3$), 3.36 (m)overlapped by OCH$_3$), 1, H$^{5'}$), 3.38 (s, 3, OCH$_3$), 3.55 (d of d, $J_{5''-5'}$ = 10.5 Hz, $J_{5''-4'}$ = 3.5 Hz, 1, H$^{5''}$), 4.24 (m, 1, H$^{4'}$), 4.38 ("t", $J_{3'-2'} \simeq J_{3'-4'} \simeq$ 5.2 Hz, 1, H$^{3'}$), 5.93 ("t", $J_{2'-3'} \simeq J_{2'-1'} \simeq$ 4.7 Hz, 1, H$^{2'}$), 6.07 (br s, 2, 6-NH$_2$), 6.16 (d, $J_{1'-2'}$ = 4.2 Hz, 1, H$^{1'}$), 7.30 (m, 15, Cϕ_3), 7.98, 8.28 (s,s; 1,1; H$_2$,H$_8$); ms (200°) m/e 322 (37, M-Cϕ_3), 306 (2.5, M-OCϕ_3), 280 (2.5, M-(Cϕ_3 + COCH$_2$)), 243 (23, Cϕ_3), 241 (10), 239 (8), 228 (11), 215 (7.5), 220 (3,x-COCH$_3$), 204 (4,y), 202 (6), 187 (23, w-Cϕ_3), 178 (7, d-COCH$_2$), 165 (100, Cϕ_3-ϕ), 164 (11, h), 152 (5), 148 (7.5 f), 139 (8), 136 (43, B + 2H), 135 (24, B + H).

The solid *19* (less 0.1 g) was dissolved in 100 ml of 80% aqueous HOAc and heated at 100° for 15 min with stirring. The pale yellow solution was poured into 300 ml of cold H$_2$O and extracted with Et$_2$O (2 x 200 ml). The combined organic phase was back-extracted with 10% HOAc (3 x 100 ml). All the aqueous layers were combined and evaporated to dryness and the product was crystallized from 98% EtOH to give 4.09 g (81%) of *20* mp 214.5 - 215.5°;

uv (MeOH) max 259 nm (ε 14,300), min 227 nm (ε 1,600); nmr (DMSO-d_6) δ 2.05 (s, 3, $OCOCH_3$), 3.35 (s, 3, OCH_3), 3.68 (m, 2, $H^{5'}$,$H^{5''}$), 4.18 (m, 2, $H^{4'}$,$H^{3'}$), 5.49 (br"t", $J_{OH-H^{5'},H^{5''}}$ = 6.0 Hz, 1, 5'-OH), 5.78 ("t", $J_{2'-1'} \approx J_{2'-3'} \approx$ 5.3 Hz, 1, $H^{2'}$), 6.15 (d, $J_{1'-2'}$ = 5.8 Hz, 1, $H^{1'}$), 7.36 (br s, 2, 6-NH_2), 8.18, 8.20 (s,s; 1,1; H^2,H^8); ms (200°) m/e 323 (2,M), 308 (0.3, M-CH_3), 306 (0.2, M-OH), 293 (6.5, c), 280 (0.3, M-$COCH_3$), 264 (5, M-$OCOCH_3$), 262 (2.5,x), 250 (0.5, c-$COCH_3$), 234 (4.5, c-$OCOCH_3$), 232 (4, M-($HOCH_3$ + $HOCOCH_3$)), 220 (24, d), 204 (12.5, y), 202 (9.5), 189 (6.0, s), 178 (16.5, d-$COCH_2$), 173 (4.5, y-OCH_3), 164 (32,h), 148 (16, f), 146 (5,s-$COCH_3$), 136 (100, B + 2H), 135 (65, B + H).

Anal. Calcd for $C_{13}H_{17}N_5O_5$: C, 48.29; H, 5.30: N, 21.66. Found: C, 48.02; H, 5.38; N, 21.70.

9-(3-*O*-Methyl-5-*O*-pivalyl-β-*D*-ribofuranosyl)adenine (*22*)

To a stirred solution of 3.9 g (0.0121 mole) of *20* (dried by repeated evaporation of dry pyridine) in 125 ml of dry pyridine, cooled in an ice bath, was added 2.3 ml (0.018 mole) of pivalyl chloride dropwise with exclusion of moisture. The solution was stirred for 18 h at 5° and poured into 300 ml of ice H_2O. The aqueous solution was extracted with $CHCl_3$ (2 x 150 ml) and the combined organic extract was washed with cold 10% $NaHCO_3$ (2 x 150 ml) and H_2O (150 ml). The $CHCl_3$ solution was evaporated to dryness and coevaporated with dry toluene (2 x 50 ml) and 98% EtOH (50 ml) to leave 5.09 g of 9-(2-O-acetyl-3-O-methyl-5-O-pivalyl-β-D-ribofuranosyl) adenine (21) as a white solid containing about 20% of 6-N-pivalamido-9-(2-O-acetyl-3-O-methyl-5-O-pivalyl-β-D-ribofuranosyl)adenine by nmr. Spectral analysis of the major compound gave; nmr ($CDCl_3$) δ 1.19 (s, 9, $OCOC(CH_3)_3$), 2.13 (s, 3, $OCOCH_3$), 3.41 (s, 3, OCH_3), 4.33 (m, 4, $H^{3'}$, $H^{4'}$, $H^{5'}$, $H^{5''}$), 5.93 (m, 3, $H^{2'}$, 6-NH_2), 6.07 (d, $J_{1',2'}$= 3.8 Hz, 1, $H^{1'}$), 7.90, 8.33 (s,s;1,1;H^2, H^8); mass spectrum (Calcd for $C_{18}H_{25}N_5O_6$: 407.1805; Found: m/e 407.1816).

The solid (less 0.1 g) was then dissolved in 125 ml of 95% EtOH and 100 ml of concentrated NH_3(aq) was added. This solution was stirred at 24° for 1.5 h, evaporated to a small volume and mixed with 150 ml of H_2O and 300 ml of $CHCl_3$. The $CHCl_3$ extracts were combined and washed with cold 10% $NaHCO_3$ (200 ml), H_2O (200 ml) and evaporated to dryness. The resulting oil was crystallized from acetone (n-pentane, desiccator) and the resulting solid was recrystallized in the same manner to give 2.42 g of pure 22. The mother liquors were again treated with concentrated NH_3 as above and after analogous workup and crystallization yielded a further 0.87 g of 22. The mother liquors from this crystallization were again treated as before to yield a further 0.18 g of 22, for a total yield of 3.47 g (78%) of 22. The pure material had mp 138 - 139°; uv (MeOH) max 259 nm (ε 13,400), min 227 nm (ε 1,800); nmr ($CDCl_3$) δ 1.17 (s, 9, $OCOC(CH_3)_3$), 3.49 (s, 3, OCH_3), 4.04 (m, 1, $H^{3'}$), 4.32 (m, 3, $H^{4'}$,$H^{5'}$,$H^{5''}$), 4.85 (m, 2, $H^{2'}$, 2'-OH), 5.95 (d, $J_{1'-2'}$ = 4.8 Hz, 1, $H^{1'}$), 6.20 (br s, 2, 6-NH_2), 7.83, 8.28 (s,s;1,1;H^2,H^8), 2.56 (br s, 0.5, H_2O); nmr ((DMSO-d_6) δ 3.41 (s, 3, OCH_3), 3.96 (m, 1, $H^{3'}$), 4.24 (m, 3, $H^{4'}$,$H^{5'}$,$H^{5''}$), 4.88 (m, 1, $H^{2'}$), 5.61 (d, $J_{OH-H^{2'}}$ = 6.5 Hz, 1, 2'-OH), 5.91 (d, $J_{1'-2'}$ = 5.2 Hz, 1, $H^{1'}$), 7.27 (br s, 2, 6-NH_2), 8.17, 8.30 (s,s; 1,1;H^2,H^8), 3.34 (s, rel. int. = 0.5, H_2O); ms (200°) m/e 365 (0.5, M), 350 (8.5, M-CH_3), 335 (0.5, M-30), 280 (0.5 M-$COC(CH_3)_3$), 264 (3, M-$OCOC(CH_3)_3$), 250 (0.5, c), 232 (1, c-H_2O), 220 (0.5, x), 206 (0.5), 192 (0.5), 190 (0.5), 178 (5.5, d), 177 (2), 174 (0.5), 164 (100,h), 149 (1.5, e), 148 (9, f), 136 (89, B + 2H), 135 (51, B + H).

Anal. Calcd for $C_{16}H_{23}N_5O_5 \cdot 0.25\ H_2O$: C, 51.95; H, 6.40; N, 18.94. Found: C, 51.80; H, 6.66; N, 19.09.

9-(2-Chloro-2-deoxy-3-O-methyl-5-O-pivalyl-β-D-arabinofuranosyl)adenine (23).

To a stirred solution of 0.5 g (0.00135 mole) of 22 (dried by repeated evaporation of dry pyridine) in ~30 ml of dry pyridine, cooled in an ice bath, was injected (through a septum) 0.5 ml (0.00695 mole) of $SOCl_2$ with exclusion of moisture. The resulting red solution was stirred for 30 min and after warming to room temperature was placed in a 120° oil bath for 1 h. After 5 min at 120°, the reaction turned black in colour. The black solution was poured into 150 ml of ice water and extracted with Et_2O (3 x 125 ml). The combined organic extracts were washed with cold 10% $NaHCO_3$ (2 x 150 ml) and H_2O (150 ml) and evaporated to dryness. Coevaporation with toluene (2 x 50 ml) removed residual pyridine. The dark brown residue was taken up in a small amount of hot 98% EtOH and filtered. On cooling a brown, non-nucleoside precipitate appeared which was also filtered off. The resulting EtOH solution was then evaporated to dryness, dissolved in a small amount of $CHCl_3$ and applied to a silica gel column (30 g) packed in $CHCl_3$. The column was then eluted with MeOAc. The first 165 ml of eluate was discarded and the following 130 ml was evaporated to dryness to give 0.109 g (21%) of crude 23 which gave 0.086 g (16.5%) of pure 23, in two crops, after crystallization from acetone (n-pentane, desiccator). This pure material had mp 185 - 187°; uv (MeOH) max 258 nm (ε 14,300), min 226 nm (ε 2,500); nmr ($CDCl_3$) δ 1.20 (s, 9, $OCOC(CH_3)_3$), 3.47 (s, 3, OCH_3), 4.26 (m, 4, $H^{3'}$,$H^{4'}$,$H^{5'}$, $H^{5''}$), 4.61 (d of d, $\underline{J}_{2',3'}$= 1.6 Hz, $\underline{J}_{2',1'}$ = 4.2 Hz, 1, $H^{2'}$), 6.21 (br s, 2, 6-NH$_2$), 6.48 (d, $\underline{J}_{1'-2'}$ = 4.2 Hz, 1, H$^{1'}$), 8.10, 8.34 (s, s; 1,1;H^2,H^8); ms (210°) m/e 383 (2.5 M), 368 (1.5, M-CH$_3$), 352 (1.5, M-OCH$_3$), 316 (1), 298 (12, M-COC(CH$_3$)$_3$), 282 (25, M-OCOC(CH$_3$)$_3$), 268 (0.5, c), 252 (2), 246 (3), 238 (1,x), 220 (2, B + H + COC(CH$_3$)$_3$), 214 (3.5, s-Cl), 204 (8.5, y), 202 (3), 196 (2.5, d), 173 (3, y-OCH$_3$), 164 (35, h), 160 (3.5), 145 (4), 136 (100, B + 2H), 135 (51, B + H).

Anal. Calcd for $C_{16}H_{22}N_5O_4Cl$: C, 50.06; H, 5.78; N, 18.25; Cl, 9.24. Found: C, 49.77; H, 5.90; N, 18.49; Cl, 9.40.

9-(2-Deoxy-3-O-methyl-β-D-ribofuranosyl)adenine (24)

To a solution of 75 ml of dry pyridine and 0.57 ml (0.00792 mole) of SOCl$_2$ in a 500 ml three necked round bottomed flask, fitted with a mechanical stirrer, dropping funnel, dry N$_2$ flow and in an ice bath, was added 0.58 g (0.00157 mole) of 22 (dried by repeated evaporations of pyridine) in 100 ml dry pyridine dropwise over 15 min. The resulting solution was stirred for an additional 10 min and the resulting red solution was then put into a 120° oil bath for 1 h. As in the other reactions, the solution turned black after about 5 min. After 1 h in the oil bath, the reaction mixture was poured into 75 ml of ice water and extracted with CHCl$_3$ (2 x 100 ml). The combined $CHCl_3$ extracts were filtered and evaporated to dryness. Coevaporation of toluene (2 x 50 ml) removed residual pyridine. The black residue was taken up in a small volume of $CHCl_3$ and absorbed on 3 g of silica gel which was applied to the top of a dry-packed silica gel column (1.6 x 36 cm, 25 g). The column was eluted with MeOH : $CHCl_3$ (1 : 19) and after the initial 25 ml of eluate was discarded, the next 50 ml was collected and evaporated to dryness.

The resulting oil was mixed with 25 ml of dry benzene, 0.01 g (0.0061 mole) of 2,2'-azobis(2-methylpropanenitrile) and 5 ml of tri-n-butyltin hydride solution in benzene (0.005 mole). This orange coloured mixture was heated at reflux for 1 h and another 0.01 g (0.00061 mole) of 2,2'-azobis(2-methylpropanenitrile) was added. After a further 2.75 h at reflux the pale yellow reaction mixture was cooled, concentrated to about one third of the volume and slowly added to 200 ml of n-pentane. The resulting precipitate was filtered off and dissolved in $CHCl_3$ and the $CHCl_3$ evaporated to leave a pale yellow amorphous solid.

This solid was dissolved in 25 ml of MeOH containing 1.25 g (0.054 mole) of Na. After stirring for 0.5 h, 30 ml of H_2O was added and the reaction was neutralized with HOAc. The MeOH was evaporated and the now aqueous layer was extracted with MeOAc (3 x 30 ml). The combined organic extract was evaporated to dryness and dissolved in 50 ml of H_2O and 50 ml of *n*-pentane. The aqueous layer was extracted with *n*-pentane, concentrated, applied to a column (2.5 x 53 cm) of Dowex 1-X2 (OH^-) resin and the column was eluted with H_2O. The first 460 ml of eluate was discarded and the following 490 ml was evaporated to give an overall yield of 0.00029 moles (18.6%) of 24 by uv analysis. This product was crystallized from 98% EtOH (*n*-pentane, desiccator) to give 0.059 g (14%) of 24.

This pure material had mp 159 - 170°; $[\alpha]_D^{24}$ - 25.4° (*c* 0.5, DMF); uv (0.1 *N* HCl) max 258 nm (ε 15,100), min 229 nm (ε 3,100); uv (H_2O) max 260 nm (ε 15,600), min 226 (ε 2,600); uv (0.1 *N* NaOH) max 260 nm (ε 15,500), min 223.5 nm (ε 1,300); nmr (DMSO-d_6) δ 2.50 (m, 1 (overlapped by $CD_3S(O)CD_2H$ peak), $H^{2''}$), 2.77 (d of d of d, $J_{2'-3'}$ = 5.0 Hz, $J_{2'-1'}$ = 8.5 Hz, $J_{2'-2''}$ = 14 Hz, 1, $H^{2'}$), 3.30 (s, 3 (from D_2O exchange since peak is overlapped by H_2O peak), OCH_3), 3.58 (m, 2, $H^{5'}$,$H^{5''}$), 4.07 (m, 3, $H^{3'}$, $H^{4'}$, 5'-OH), 6.26 (d of d, $J_{1'-2'}$ = 8.5 Hz, $J_{1'-2''}$ = 6.5 Hz, 1, $H^{1'}$), 7.36 (br s, 2, 6-NH_2), 8.14, 8.33 (s,s;1,1; H^2, H^8), 3.30 (s, rel. int. = 0.5, H_2O); ms (Calcd for $C_{11}H_{15}N_5O_3$: 265.1175; Found: m/e 265.1172) (150°) m/e 265 (5, M), 250 (9, M-CH_3), 235 (11.5 c), 234 (3.5, M-OCH_3), 204 (3, x), 190 (2.5, j), 165 (4.5, h), 162 (38, d), 145 (2), 136 (37, B + 2H), 135 (100, B + H), 131 (6, s).

Anal. Calcd for $C_{11}H_{15}N_5O_3 \cdot 0.25\ H_2O$: C, 48.97; H, 5.79; N, 25.88. Found: C, 48.89; H, 5.85; N, 25.88.

Preparation of 9-(2-Deoxy-β-D-erythro-pentofuranosyl)-adenine[2'-deoxyadenosine] (10) and 9-(3-Deoxy-β-D-erythro-pentofuranosyl)adenine[3'-deoxyadenosine;cordycepin] (31).

To a stirred solution of 19.3 g (0.059 moles) of 9-(2,3-O-methoxyethylidene-β-D-ribofuranosyl)adenine (25)[144,145] (dried by repeated evaporation of dry pyridine) in 100 ml of dry pyridine, cooled in a salt-ice bath, was added 0.05 g (0.0004 mole) of 4-dimethylaminopyridine and 8 ml (0.085 mole) of acetic anhydride, with exclusion of moisture. After stirring for 50 min, MeOH (50 ml) was added and the reaction was stirred for an additional 30 min and evaporated to dryness. Toluene, 98% EtOH, and CH_2Cl_2 were coevaporated several times to remove residual solvent. An nmr spectrum (DMSO-d_6) of 0.05 g of the residual amorphous solid (20.6 g) showed the desired 9-(5-O-acetyl-2,3-O-methoxyethylidene-β-D-ribofuranosyl)adenine (26) in a diastereomeric ratio (due to methoxyethylidene) of 3 : 2. A mass spectrum contained the expected molecular ion: (Calcd for $C_{15}H_{19}N_5O_6$: 365.1335; Found 365.1331).

This crude material was dissolved in 5% HOAc (200 ml) and 98% EtOH (10 ml) and stirred at 24° for 1 h.[145] The reaction mixture was then evaporated to dryness and

98% EtOH and toluene were coevaporated several times to remove residual acid. An nmr spectrum (DMSO-d_6) of 0.077 g of the resulting amorphous solid (22.37g) showed: 9-(3,5-di-*O*-acetyl-β-*D*-ribofuranosyl)adenine (27) δ 5.05 ("t", $J_{2'-1'} \approx J_{2'-3'}$ = 5.8 Hz, 1, $H^{2'}$), 5.16 (m, 1, $H^{3'}$), 5.95 (d, $J_{1'-2'}$ = 5.9 Hz, 1, $H^{1'}$) and 9-(2,5-di-*O*-acetyl-β-*D*-ribofuranosyl)adenine (28) δ 4.65 ("t", $J_{3'-2'} \approx J_{3'-4'} \approx$ 5.7 Hz, 1, $H^{3'}$), 5.77 ("t", $J_{2'-3'} \approx J_{2'-1'} \approx$ 5.0 Hz, 1, $H^{2'}$), 6.17 (d, $J_{1'-2'}$ = 4.2 Hz, 1, $H^{1'}$). The mixture was ~37% 28 and 63% 27. A mass spectrum contained the expected molecular ion (Calcd for $C_{14}H_{17}N_5O_6$: 351.1179; Found: 351.1176).

A 0.96 g (0.0025 mole) sample of this mixture was dryed by repeated evaporation of dry pyridine and dissolved in ~100 ml of dry pyridine. This solution was cooled to 4° and added dropwise to a solution of 1.1 ml (0.0153 mole) of $SOCl_2$ in 75 ml of dry pyridine which was mechanically stirred in an ice bath under a flow of dry N_2 and exclusion of moisture. Stirring was continued for 20 min. The solution was then stirred vigorously in an oil bath for 30 min at 120°. The resulting black solution was poured into 50 ml of ice water and extracted with CH_2Cl_2 (3 x 75 ml). The organic extracts were evaporated to dryness and toluene was coevaporated several times to remove residual pyridine. The residue was adsorbed on 3 g of silica gel and the resulting dark brown powder applied to a silica gel column (1.8 x 20 cm, 25 g). The column was eluted with MeOH: CH_2Cl_2 (1 : 19). After the first 40 ml of eluate was discarded, the next 70 ml, containing product slightly contaminated with starting material, was collected and evaporated to dryness. A further 480 ml of eluate containing starting material was collected and evaporated to dryness. Uv spectroscopy indicated that 0.00075 mole of product (mixture of 2' and 3' chloro compounds) and 0.001 mole of starting material had been collected. The starting material was dissolved in CH_2Cl_2 and precipitated upon slow addition to n-pentane to yield 0.350 g of solid material. The yield of mono chlorinated product, less recovered starting material, was 49%.

An nmr spectrum ($CDCl_3/D_2O$) of the product gave a ratio of 9-(2-chloro-2-deoxy-3,5-di-O-acetyl-β-D-arabinofuranosyl)adenine (29) : 9-(3-chloro-3-deoxy-2,5-di-O-acetyl-β-D-xylofuranosyl)adenine (30) of ~3 : 2 (δ 6.52 (d, $J_{1'-2'}$ = 3.9 Hz, 1, $H^{1'}$) : 6.24 (d, $J_{1'-2'}$ = 2.2 Hz, 1, $H^{1'}$)). A mass spectrum of this mixture contained the expected molecular ions (Calcd for $C_{14}H_{16}N_5O_5\,{}^{35}Cl$: 369.0841; Found: 369.0841).

The total product (0.00075 mole) was dissolved in dry benzene (10 ml) and 0.025 g (0.00152 mole) of 2,2'-azobis(2-methyl-propanenitrile) and 12.5 ml of tri-n-butyltin hydride solution in benzene (0.0125 mole) was added with stirring and exclusion of moisture. This mixture was heated at reflux for 4 h, concentrated to ~5 ml and precipicated upon slow addition to n-pentane (400 ml). A mass spectrum of the precipitate contained the expected molecular ions (Calcd for $C_{14}H_{17}N_5O_5$: 335.1230; Found 335.1227).

The precipitate was dissolved in 98% EtOH (25 ml) and concentrated aqueous NH_3 (10 ml) and stirred for 23 h at room temperature. The solution was evaporated to dryness, dissolved in a small amount of H_2O and applied to a Dowex 1-X2 (OH^-) column (72 x 2.2 cm). The column was eluted with 4.0 l of H_2O, 2.0 l of MeOH : H_2O (1 : 9), 1.6 l of MeOH : H_2O (3 : 7), 1.5 l of MeOH : H_2O (6 : 4) and 2.0 l of MeOH : H_2O (9 : 1). After the first 3.5 l of eluate was discarded, 1.5 l of eluate containing pure 10 was collected. A further 1.1 l of uv transparent eluate was discarded and then 0.75 l of eluate containing pure 31 was collected. A further 2.5 l of uv transparent eluate was discarded and then 0.7 l of eluate containing adenosine was collected. Analysis by uv showed 0.0004 mole (26.6%, based on recovered starting material) of 10, 0.00027 mole (18%, based on recovered starting material) of 31 and 0.000047 mole of adenosine. The solution containing pure 10 was evaporated to dryness and the residue was crystallized from MeOH (ether, desiccator) to give

0.067 g (17.5%, based on recovered starting material) of *10*. This material had mp 193.5 - 194°; $[\alpha]_{D}^{24}$- 27.3° (*c* 0.7, H_2O); uv (H_2O) max 260 nm (ε 15,200), min 226.5 nm (ε 2,200); uv (0.1 *N* HCl) max 257.5 nm (ε 14,500), min 228 nm (ε 2,800); uv (0.1 *N* NaOH) max 260 nm (ε 15,200), min 227 nm (ε 2,700); nmr (DMSO-d_6) δ 2.26 (d of d of d, $J_{2'-2''}$ = 13.1 Hz, $J_{2'-1'}$ = 6.0 Hz, $J_{2'-3'}$ = 2.9 Hz, 1, $H^{2'}$), 2.75 (d of d of d, $J_{2''-2'}$ = 13.1 Hz, $J_{2''-1'}$ = 7.8 Hz, $J_{2''-3'}$ = 5.8 Hz, 1, $H^{2''}$), 3.58 (m, 2, $H^{5'}$,$H^{5''}$), 3.89 (m, 1, $H^{4'}$), 4.42 (m, 1, $H^{3'}$), 5.26 (m, 2, 3-OH, 5-OH), 6.34 (d of d, $J_{1'-2'}$ = 6.0 Hz, $J_{1'-2''}$ = 7.8 Hz, 1, $H^{1'}$), 7.27 (br s, 2,6-NH_2), 8.13, 8.34 (s,s;1,1; H^2, H^8); ms (200°) m/e 251 (2.5, M), 234 (0.4, M-OH), 233 (0.5, M-H_2O), 221 (4.5, c), 220 (0.8, M-OCH_3), 204 (0.4, x), 191 (2.0), 190 0.6, j), 179 (0.6), 178 (0.3, i), 164 (14, h), 162 (38, e), 161 (2.0), 149 (0.8, e), 148 (0.8, f), 145 (1.0), 136 (29, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.87. Found: C, 47.78; H, 5.29; N, 27.94.

The eluate containing pure *31* was evaporated to dryness and crystallized from 98% EtOH (Et_2O, desiccator) to give, in two crops, 0.043 g (11% based on recovered starting material) of *31*. This material had mp 210 - 211.5°; $[\alpha]_{D}^{24}$- 46.5° (*c* 0.75, H_2O); uv (H_2O) max 260 nm (ε 14,600), min 227 (ε 22 ,100); uv (0.1 *N* HCl) max 258 nm (ε 14,500), min 230 nm (ε 3,300); uv (0.1 *N* NaOH) max 260 nm (ε 14,500), min 228 nm (ε 2,500); nmr (DMSO-d_6) 1.93 (d of d of d, $J_{3'-3''}$ = 12.9 Hz, $J_{3'-4'}$ = 6.5 Hz, $J_{3'-2'}$ = 3.5 Hz, 1, $H^{3'}$), 2.28 (d of d of d, $J_{3''-3'}$ = 12.9 Hz, $J_{3''-4'}$ = 8.3 Hz, $J_{3''-2'}$ = 5.9 Hz, 1, $H^{3''}$), 3.61 (m, 2, $H^{5'}$, $H^{5''}$), 4.37 (m, 1, $H^{4'}$), 4.59 (m, 1, $H^{2'}$), 5.16 (br "t", $J_{OH-5'}$ ≃ 5.0 Hz, 1, 5'-OH), 5.66 (br d, $J_{OH-2'}$ = 4.2 Hz, 1, 2'-OH), 5.88 (d, $J_{1'-2'}$ = 2.2 Hz, 1, $H^{1'}$), 7.27 (br s, 2,6-NH_2), 8.16, 8.36 (s,s;1,1; H^2, H^8); ms (185°) m/e 251 (4.5, M), 234 (1, M-OH), 233 (0.6 M-H_2O), 221 (11.5, c), 220 (6, x), 216 (1), 215 (2), 204 (1.5, c-OH), 203 (2, c-H_2O), 202 (3.5), 193 (1), 192 (2), 178 (19, d), 175 (3.5), 164 (71, h), 149 (2.5, e), 148 (9.5, f), 136 (48, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.87. Found: C, 47.57; H, 5.26; N, 27.89.

Evidence for arabino configuration for 29 and xylo configuration for 30

To a crude mixture of ~0.075 g (0.0002 moles) of 29 : 30 (~3 : 2, prepared as given in the above procedure) was added 98% EtOH (10 ml) and concentrated aqueous NH_3 (5 ml). This solution was stirred for 3 h and the reaction evaporated to dryness. EtOH (98%) was added and coevaporated (4 x 20 ml) to give a yellow amorphous solid which was applied to a silica gel column (1.4 x 17 cm, 35 g) wet packed in $CHCl_3$. The column was eluted with MeOH : $CHCl_3$ (1 : 9). The first 25 ml of eluate was discarded and the next 15 ml was collected. The following 12 ml was discarded and the next 20 ml was collected.

The first 15 ml collected was evaporated to dryness and proved to be 9-(3-chloro-3-deoxy-β-D-xylofuranosyl)adenine (55) containing a small amount of 9-(2,3-anhydro-β-D-ribofuranosyl)adenine (56) (epoxide formation could be prevented by deblocking with MeOH saturated with NH_3 and kept at 0° or lower).[167] This material had nmr (DMSO-d_6) δ 3.75 (m, 2, $H^{5'}$,$H^{5''}$), 4.48 (m, 2, $H^{4'}$,$H^{3'}$), 4.79 (m, 1, $H^{2'}$), 5.28 (br s, 1, OH), 5.87 (d, $\underline{J}_{1'-2'}$ = 4.0 Hz, 1, $H^{1'}$), 6.36 (br s, 1, OH), 7.30 (br s, 2, 6-NH_2), 8.16, 8.25 (s,s; 1,1; H^2,H^8) which was identical to an authentic sample.[135]

The second amount collected (20 ml) was evaporated to dryness and proved to be 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)adenine (57). Some material that crystallized directly from the column eluate had mp of 242.5 - 243° and was not depressed when mixed with an authentic sample of 57.[170] This material had nmr (DMSO=$\underline{d}_6$) δ 3.77 (m, 3, $H^{5'}$,$H^{5''}$,$H^{4'}$), 4.49 (m, 1, $H^{3'}$), 4.75 (d of d, $\underline{J}_{2'-1'}$= 6.2 Hz, $\underline{J}_{2'-3'}$ = 7.5 Hz, 1, $H^{2'}$), 5.22 (br s, 1, OH), 6.08 (br s, 1, OH), 6.49 (d, $\underline{J}_{1'-2'}$ = 6.2 Hz, 1, $H^{1'}$), 7.27 (br s, 1, 6-NH_2), 8.16, 8.36 (s, s; 1,1; H^2,H^8); ms (200°) m/e 285 (1.5, M), 268 (1, M-OH), 255 (2,c), 250 (3.5, M-Cl), 233 (1), 220 (8,c-Cl), 218 (1), 213 (2, i +H), 202 (1.5), 196 (3.5, d), 190 (5, j), 178 (1.5), 173 (1.5), 164 (47, h), 162 (3), 161 (2), 160 (2.5), 149 (1,e), 148 (2, f), 145 (1), 136 (92, B + 2 H), 135 (100, B + H) which is identical to an authentic sample.[135]

Both chloro samples (55 and 57) could be converted to the epoxide (56) by treatment witn NaOMe in MeOH. This further confirms their trans configuration at the 2' and 3' positions.

9-(2-O-Methyl-5-O-triphenylmethyl-β-D-ribofuranosyl)adenine (33)

To a stirred solution of 16.9 g (0.0601 mole) of 9-(2-O-methyl-β-D-ribofuranosyl)adenine (dried by repeated addition and evaporation of dry pyridine) in ~200 ml of dry pyridine was added 20.1 g (0.072 mole) of triphenylmethyl chloride. This solution was heated to 100° for 1 h with exclusion of moisture and then poured into a stirred mixture of 300 ml of $CHCl_3$ and 300 ml of cold 10% $NaHCO_3$. The organic layer was separated and the aqueous layer extracted again with $CHCl_3$ (100 ml). The combined organic extracts were washed with cold H_2O (100 ml). The aqueous layers were combined and extracted with a further 50 ml of $CHCl_3$. All the organic extracts were combined and evaporated to dryness and coevaporation of the residue with toluene (2 x 100 ml) removed residual pyridine.

The aqueous layers were collected, concentrated to 300 ml and extracted continuously with CH_2Cl_2 to give back 1.56 g (0.0055 mole) of starting material. The residue from the evaporated organic phase was taken up in a small volume of CH_2Cl_2 and applied to a silica gel (Begr. Hermann, Kieselgel) column (7.5 x 61 cm, 1000 g) packed in CH_2Cl_2. The column was eluted with CH_2Cl_2 (8.7 l) and then MeOH : CH_2Cl_2 (1 : 24). The first 0.7 l of eluate was discarded and the next 0.8 l of contaminated product was collected. A further 2.5 l was collected, evaporated to dryness, dissolved in CH_2Cl_2 (100 ml) and precipitated from *n*-pentane (1.8 l) to give 14.5 g of **33**. The *n*-pentane solution was evporated to dryness and the residue reprecipitated to give an additional 0.45 g of **33**.

The 0.8 l of impure product was purified by similar chromatography on a second column (700 g) to yield an additional 7 g of **33**. The total product was collected and dried *in vacuo* over $CaSO_4$ and paraffin, to yield 20.0 g (0.0382 mole) of **33**. The overall yield was 70% based on recovered starting material.

This product was crystallized from Et_2O to give crystals with mp 117 - 118°; uv (MeOH) max 258 nm (ε 15,400), min 240 nm (ε 8700); nmr (DMSO-d_6) δ 3.37 (m, 5 (overlapped by H_2O peak), OCH_3, $H^{5''}$, $H^{5'}$), 4.08 (m, 1, $H^{4'}$), 4.47 (m, 2, $H^{3'}$, $H^{2'}$), 5.27 (br s, 1, 3'-OH), 6.07 (d, $J_{1'-2'}$ = 3.4 Hz, 1, $H^{1'}$), 7.29 (m, 17, $C\phi_3$, 6-NH_2), 8.12, 8.28 (s,s; 1,1; H^2, H^8); ms (230°) m/e 523 (0.5, M), 388 (0.5, w), 376 (1.5), 280 (100, M-$C\phi_3$), 264 (14, M-$OC\phi_3$), 250 (2, c-$C\phi_3$), 243 (100, $C\phi_3$), 228 (6), 215 (3.5), 202 (2), 192 (12, d), 183 (1.5), 177 (2,w), 176 (2), 165 (56, $C\phi_3$-ϕ), 148 (4, f), 145 (4, w-$C\phi_3$), 136 (44, B + 2H), 135 (10, B + H).

Anal. Calcd for $C_{30}H_{29}N_5O_4$: C, 68.85; H, 5.58; N, 13.38. Found: C, 68.91: H, 5.89: N, 13.35.

9-(2-O-Methanesulfonyl-3-O-methyl-β-D-ribofuranosyl)adenine (34).

To a stirred solution of 0.5 g (0.00096 mole) of 18 (dried by repeated addition and evaporation of dry pyridine) in ~10 ml of dry pyridine, cooled in an ice bath, was added 0.15 ml (0.00195 mole) of methanesulfonyl chloride dropwise with exclusion of mixture. After the addition was complete, the reaction was allowed to warm to 24° and stirred for a further 1.25 h. The resulting solution was poured into cold saturated $NaHCO_3$ (50 ml) and extracted with $CHCl_3$ (2 x 50 ml). The combined $CHCl_3$ layers were washed with H_2O (50 ml) and evaporated to dryness. Coevaporation of toluene (2 x 50 ml) removed residual pyridine.

The resulting amorphous solid was dissolved in 80% HOAc (30 ml) and heated to 100° for 15 min in an oil bath. After cooling, 120 ml of cold H_2O was added and the aqueous solution extracted with Et_2O (2 x 60 ml).

The combined organic extracts were back-extracted with 10% HOAc (3 x 40 ml). The combined acidic, aqueous layers were evaporated to dryness and residual water and acid removed by several coevaporations with 98% EtOH and Et_2O. The remaining solid was crystallized from H_2O to give 0.283 g (83%) of pure **34**; mp 226.5 - 228.5°; $[\alpha]_{\underline{D}}^{24}$ -48.0 (*c* 1.2, DMF); uv (MeOH : H_2O [1 :9]) max 258 nm (ε 15,300), min 224 nm (ε 2200); uv (MeOH : 0.1 *N* HCl [1:9]) max 256 nm (ε 15,400), min 226 nm (ε 3000); uv (MeOH: 0.1 *N* NaOH [1:9]) max 258 nm (ε 15,200), min 224 nm (ε 2500); uv (0.1 *N* HCl) max 256 nm (ε 15,200), min 226 nm (ε 3100); nmr (DMSO-$\underline{d}_6$) δ 3.24, 3.43 (s,s; 3,3; OCH_3, SO_2CH_3), 3.71 (m, 2 $H^{5'}$, $H^{5''}$), 4.13 (m, 1, $H^{4'}$), 4.18 ("t", $\underline{J}_{3'-2'} \simeq \underline{J}_{3'-2'} \simeq 4.7$ Hz, 1, $H^{3'}$), 5.50 ("t", $\underline{J}_{OH-5'5''} \simeq 5.0$ Hz, 1, 5' - OH), 5.72 ("t", $\underline{J}_{2'-3'} \simeq \underline{J}_{2'-1'} \simeq 5.0$ Hz, 1, $H^{2'}$), 6.15 (d, $\underline{J}_{1'-2'} = 4.8$ Hz, 1, $H^{''}$), 7.37 (br s, 2, 6-NH_2), 8.16, 8.38 (s, s; 1, 1; H^2, H^8); ms (160°) m/e 359 (3.5, M), 344 (0.15, M-CH_3), 342 (0.15, M-OH), 329 (8.5, c), 328 (2, M-OCH_3), 298 (2.5, x), 280 (5.5, M-SO_2CH_3), 263 (3, M-$HOSO_2CH_3$), 256 (14, d), 234 (8, c-OSO_2CH_3), 232 (5, M-(OCH_3 + $HOSO_2CH_3$)) 220 (s, x- SO_2CH_2), 204 (37, y), 202 (20), 194 (3.5, y - CH_3 or i-SO_2CH_2), 190 (3, j), 177 (10, d-SO_2CH_3), 173 (5, y-OCH_3), 164 (58, h), 163 (16), 148 (17, f), 145 (4.5, w-SO_2CH_3), 136 (96, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{12}H_{17}N_5O_6S$: C, 40.10; H, 4.77; N, 19.49; S, 8.92. Found: C, 39.96; H, 4.82; N, 19.51; S, 8.94.

9-(3-*O*-Methanesulfonyl-2-*O*-methyl-β-D-ribofuranosyl)adenine (35)

This compound was prepared from 33 using exactly the same procedure as for 34.

Crystallization from H_2O gave 0.257 g (75%) of pure 35; mp 229 - 231°; $[\alpha]_D^{24}$ - 69.2° (*c* 0.97, DMF); uv (MeOH : H_2O [1:9]) max 258 nm (ε 15,300), min 224 nm (ε 2500); uv (MeOH : 0.1 *N* HCl [1:9]) max 256 nm (ε 15,500), min 227 (ε 4300); uv (MeOH : 0.1 *N* NaOH [1:9]) max 258 nm (ε 15,000), min 224 nm (ε 2100); uv (0.1 *N* HCl) max 256 nm (ε 15,500), min 227 nm (ε 3400); nmr (DMSO-d_6) δ 3.32 (s, 6, OCH_3, SO_2CH_3), 3.71 (m, 2, $H^{5'}$, $H^{5''}$), 4.37 (m, 1, $H^{4'}$), 4.79 (d of d, $J_{2'-1'}$ = 7.1 Hz, $J_{2'-3'}$ = 5.0 Hz, 1, $H^{2'}$), 5.41 (d of d, $J_{3'-2'}$ = 5.0 Hz, $J_{3'-4'}$ = 1.2 Hz, 1, $H^{3'}$), 5.72 ("t", $J_{OH-5',5''}$ ≃ 5.8 Hz, 1, 5'-OH), 6.03 (d, $J_{1'-2'}$ = 7.1 Hz, 1, $H^{1'}$), 7.37 (br s, 2, 6-NH_2), 8.16, 8.39 (s, s; 1, 1; H^2, H^8); ms (170°) m/e 359 (5, M), 344 (0.2, M-CH_3), 329 (2, c), 328 (0.4, M-OCH_3), 280 (25, M-SO_2CH_3), 264 (12.5, M-OSO_2CH_3), 263 (2, M-$HOSO_2CH_3$), 248 (1.5), 234 (22,x), 233 (26, c-$HOSO_2CH_3$), 232 (s, M-(OCH_3 + $HOSO_2CH_3$)), 224 (5.5, w), 218 (2), 204 (3.5, y), 202 (18), 193 (6), 192 (10.5, d), 177 (2, v), 176 (2, s-(OCH_3 + H_2O)), 174 (3), 173 (4.5, y-OCH_3), 164 (38, h), 148 (7.5, f), 136 (74, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{12}H_{17}N_5O_6S$: C, 40.10; H, 4.77; N, 19.49; S, 8.92. Found C, 39.84; H, 4.60; N, 19.25; S, 8.88.

9-(3-O-Methyl-2-O-p-toluenesulfonyl-β-D-ribofuranosyl) adenine (38).

To a stirred solution of 0.5 g (0.00096 mole) of 18 (dried by evaporation using dry pridine) in ~10 ml of dry pyridine was added 0.276 g (0.00144 mole) of p-toluenesulfonyl chloride with exclusion of moisture. The solution was stirred for 6 days at 24° over which period further quantities [2 x 0.050 g (0.00052 mole)] of p-toluenesulfonyl chloride were added. The reaction was then poured into cold saturated $NaHCO_3$ solution (50 ml) and extracted with $CHCl_3$ (2 x 60 ml). The combined organic extracts were washed with H_2O (2 x 60 ml) and evaporated to dryness. Toluene and 98% EtOH were coevaporated from the residue several times to remove residual pyridine.

The resulting amorphous solid was dissolved in 80% aqueous HOAc (30 ml) and heated to 100° for 15 min in an oil bath. The acid solution was diluted with H_2O (120 ml) and extracted with n-pentane (5 x 80 ml). The aqueous solution was evaporated to dryness and toluene coevaporated several times to remove residual HOAc. The residue was crystallized from 98% EtOH (Et_2O, desiccator) to give 0.29 g (70%) of 38. A further 7 to 10% yield could be obtained by applying the mother liquor to a silica gel plate which was developed in MeOAc and the appropriate band eluted.

This material had mp 241-242.5°; $[\alpha]_D^{24}$ - 108° (*c* 1.04, DMF); uv (MeOH : H_2O [1:9])max 230, 260 nm (ε 12,900; 12,100), min 221, 243 nm (ε 10,900; 6700); uv (MeOH : 0.1 *N* HCl [1:9])max 229,257 nm (ε 13,200; 12,300), min 222, 243 nm (ε 12,800; 8400); uv (MeOH : 0.1 *N* NaOH [1:9]) max 230, 260 nm (ε 12,400; 12,000), min 221, 243 nm (ε 10,600; 7700); uv (0.1 *N* HCl) max 229,257 nm (ε 12,700; 12,700), min 221, 243 nm (ε 12,000; 8500); nmr (DMSO-d_6) δ 2.16 (s, 3, *p*-CH_3), 3.44 (s, 3, OCH_3), 3.60 (m, 2, $H^{5'}$, $H^{5''}$), 4.11 (m, 2, $H^{3'}$, $H^{4'}$), 5.56 (d of d, $J_{2'-1'}$ = 7.5 Hz, $J_{2'-3'}$ = 5.2 Hz, 1, $H^{2'}$), 5.78 (d of d, $J_{OH-5'}$ = 7.5 Hz, $J_{OH-5''}$ = 4.6 Hz, 1, 5'-OH), 6.06 (d, $J_{1'-2'}$ = 7.5 Hz, 1, $H^{1'}$), 7.02 (d, *J* *meta* H- *ortho* H = 8.5 Hz, 2, aromatic *meta* H), 7.37 (d, *J* *ortho* H-*meta* H = 8.5 Hz, 4, aromatic *ortho* H, 6-NH_2), 7.97, 8.14 (s,s; 1,1; H^2, H^8); ms (195°) m/e 435 (2.5, M), 420 (0.1, M-CH_3), 418 (0.15, M-OH), 405 (15.5, c), 404 (1.5, M-OCH_3), 3.86 (0.25, M-(OCH_3 + H_2O)), 374 (4.5, x), 332 (14.5, d), 306 (0.4), 301 (0.2, s), 290 (0.5), 280 (12, M-$SO_2\phi CH_3$), 268 (2), 264 (11, M-$OSO_2\phi CH_3$), 263 (3.5, M-$HOSO_2\phi CH_3$), 240 (8.5, c-$SO_2\phi CH_3$), 234 (15.5, c-$OSO_2\phi CH_3$), 232 (6.5, M-($HOSO_2\phi CH_3$ + OCH_3)), 205 (21), 204 (24, y), 202 (22), 194 (4, i-$SO_2\phi CH_2$), 190 (3.5, j), 178 (7.5, d-$SO_2\phi CH_2$), 173 (5, y-OCH_3), 164 (63, h), 155 (50, $SO_2\phi CH_3$), 148 (11, f), 139 (6, $SO\phi CH_3$), 136 (100, B + 2H), 135 (67, B + H).

Anal. Calcd for $C_{18}H_{21}N_5O_6S$: C, 49.64; H, 4.86; N, 16.08; S, 7.36. Found: C, 49.40; H, 4.92; N, 15.86; S, 7.39.

9-(2-O-Methyl-3-O-p-toluenesulfonyl-β-D-ribofuranosyl)-adenine (39).

To a stirred solution of 0.5 g (0.00096 mole) of 33 (dried by evaporation using dry pyridine) in ~10 ml of dry pyridine was added 0.276 g (0.00144 mole) of p-toluenesulfonyl chloride with exclusion of mixture. The solution was stirred for 7 days at 24° with one addition of 0.19 (0.00052 mole) of p-toluenesulfonyl chloride after 3 days. After this period, the reaction was poured into cold saturated $NaHCO_3$ solution (50 ml) and extracted with $CHCl_3$ (2 x 60 ml). The combined $CHCl_3$ extracts were washed with H_2O (2 x 60 ml) and evaporated to dryness. Toluene and EtOH were coevaporated from the residue several times to remove residual pyridine.

The yellow amorphous solid was dissolved in 80% HOAc (30 ml) and heated to 100° for 25 min in an oil bath. The acid solution was diluted with H_2O (120 ml) extracted with n-pentane (5 x 80 ml) and evaporated to dryness. Toluene was coevaporated several times to remove residual HOAc. The residue was crystallized from Et_2O (n-pentane, desiccator) to give 0.222 g (54%) of 39. An additional 0.030 g (61%, total) of pure 39 was obtained by application of the mother liquor to a silica plate which was developed in MeOAc and the appropriate band eluted.

This material had mp 182-184°; $[\alpha]_{\mathrm{D}}^{24}$ - 58.9° (*c* 0.64) DMF); uv (MeOH : H_2O [1:9]) max 228, 259 nm (ε 15,800; 15,400), min 220, 242 nm (ε 14,200; 9500); uv (MeOH : 0.1 *N* HCl [1:9]) max 227, 256 nm (ε 16,400; 14,700), min 220, 242 nm (ε 15,700; 10,100); uv (MeOH : 0.1 *N* NaOH [1:9]) max 228, 259 nm (ε 16,000; 14,900), min 242 nm (ε 9000); nmr (DMSO-d_6) δ 2.43 (s, 3, *p*-CH_3), 3.11 (s, 3, OCH_3), 3.51 (m, 2, $H^{5'}$, $H^{5''}$), 4.10 (m, 1, $H^{4'}$), 4.70 (d of d, $J_{2'-1'}$ = 7.1 Hz, $J_{2'-3'}$ = 5.4 Hz, 1, $H^{2'}$), 5.32 (d of d, $J_{3'-2'}$ = 5.4 Hz, $J_{3'-4'}$ = 2.0 Hz, 1, $H^{3'}$), 5.54 (br s, 1, 5'-OH), 6.01 (d, $J_{1'-2'}$ = 7.1 Hz, 1, $H^{1'}$), 7.38 (br s, 2, 6-NH_2), 7.52 (d, *J* *meta* H-*ortho* H = 8.5 Hz, 2, aromatic *meta* H), 7.92 (d, *J* *ortho* H-*meta* H = 8.5 Hz, 2, aromatic *ortho* H), 8.16, 8.37 (s,s; 1,1; H^2, H^8); ms (205°) m/e 435 (1, M), 420 (0.1, M-CH_3), 405 (0.5, c), 404 (0.5, M-OCH_3), 390 (0.6, c-CH_3), 306 (0.5, B + $HOSO_2\phi CH_3$), 300 (3.5, v), 290 (0.6, B + H + $SO_2\phi CH_3$), 289 (0.6, B + $SO_2\phi CH_3$), 280 (46, M-$SO_2\phi CH_3$), 269 (3.5, v-OCH_3), 264 (21, M-$OSO_2\phi CH_3$), 234 (26, x), 233 (19.5, c-$HOSO_2\phi CH_3$), 232 (3.5, M-($HOSO_2\phi CH_3$ + OCH_3)) 204 (2, y), 202 (12), 192 (10.5, d), 175 (3), 173 (3.5, y-OCH_3), 164 (41, h), 155 (20, $SO_2\phi CH_3$), 148 (5.5, f), 146 (4, s-$SO_2\phi CH_3$), 139 (4, $SO\phi CH_3$), 136 (62, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{18}H_{21}N_5O_6S$: C, 49.64; H, 4.86; N, 16.08; S, 7.36. Found: C, 49.77; H, 4.99; N, 16.09; S, 7.28.

9-(3-O-Methyl-2-O-p-nitrobenzenesulfonyl-β-D-ribofuranosyl)-adenine (40)

To a stirred solution of 0.5 g (0.00096 mole) of 18 (dried by evaporation using dry pyridine) in ~15 ml of dry pyridine, in an ice bath, was added 0.232 g (0.00105 mole) of p-nitrobenzenesulfonyl chloride with exclusion of moisture. The reaction was stirred for 24 h at 2° and poured into a stirred mixture of cold saturated $NaHCO_3$ (75 ml) and Et_2O (70 ml). The organic layer was separated and the aqueous layer extracted with a further 60 ml of Et_2O. The combined organic layers were evaporated to dryness and co-evaporated using toluene (2 x 50 ml) to remove residual pyridine.

The resulting yellow amorphous solid was dissolved in 80% aqueous HOAc (30 ml) and heated for 25 min at 100°. The solution was cooled and 30 ml of H_2O was added. The aqueous solution was extracted with n-pentane (4 x 60 ml) and evaporated to dryness. Residual acid was removed by coevaporation with toluene. Crystallization from isopropanol-H_2O resulted in 0.311 g (71%) of product contaminated with a trace impurity. Recrystallization from isopropanol - H_2O resulted in 0.251 g (58%) of pure 40.

This material had mp 236 - 238.5°: $[\alpha]^{24}_{D}$ - 90.9° (*c* 0.79. DMF): uv (MeOH : H_2O [1:9]) max 252 nm (ε 21.700). min 222 nm (ε 7100): uv (MeOH : 0.1 *N* HCl [1:9]) max 252 nm (ε 21.900), min 224 nm (ε 7600); uv (0.1 *N* HCl) max 253 nm (ε 22,000), min 224 nm (ε 6900); nmr (DMSO-d_6) δ 3.39 (s, 3, OCH_3), 3.62 (m, 2, $H^{5'}$, $H^{5''}$), 4.14 (m, 2, $H^{3'}$, $H^{4'}$), 5.58 ("t", $J_{OH-5',5''}$ = 6.2 Hz, 1, 5'-OH), 5.73 ("t", $J_{2'3'} \simeq J_{2'-1'} \simeq$ 6.2 Hz, 1, $H^{2'}$), 6.09 (d, $J_{1'-2'}$ = 6.2 Hz, 1, $H^{1'}$). 7.28 (br s, 2, 6-NH_2), 7.89 (d, J *ortho-meta* = 4.8 Hz, 2, aromatic protons), 7.97, 8.18 (s,s: 1,1; H^2, H^8), 8.13 (d, J *ortho-meta* = 4.8 Hz, 2, aromatic protons): ms (215°) m/e 466 (2, M). 436 (10.5, c). 435 (2, M-OCH_3). 405 (2.5. x). 363 (15, d), 299 (2.5), 280 (9. M-$HOSO_2\phi NO_2$). 270 (8), 264 (6.5, M-$OSO\phi NO_2$). 263 (8. M-$HOSO_2\phi NO_2$), 262 (3.5), 248 (2). 234 (24, c-$OSO_2\phi NO_2$), 232 (7.5, M-(OCH_3 + $HOSO_2\phi NO_2$)), 220 (4), 204 (55, y), 202 (34), 194 (6.5), 190 (6, j), 186 (19.5, $SO_2\phi NO_2$), 178 (15), 173 (9.5, y-OCH_3), 164 (81, h), 148 (21, f), 136 (100, B + 2H), 135 (90, B + H).

Anal. Calcd for $C_{17}H_{18}N_6O_8S$: C, 43.77; H, 3.89; N, 18.02; S, 6.88. Found: C, 43.58; H, 3.96; N, 18.06; S, 6.82.

Stability of 40 in base analogous with 41 and 44

A solution of 4.19 mg (0.009 mmole) of 40 in 25 ml of spectral grade MeOH was prepared. To 10 ml of this solution was added 90 ml of H_2O. The uv spectrum of this solution was determined using 2 ml giving λ max of 251.5 nm (ε 21,000). A 2 ml portion of 5.0 N NaOH was added to the original 98 ml of H_2O/MeOH (9:1) solution and again 2 ml of the now basic solution was removed for a uv determination. This uv spectrum had λ max = 256 nm (ε 24,500). A 9 ml portion of the 98 ml basic stock solution was mixed with 1.0 ml of 2.0 N HCl. The uv spectrum of this acid solution had λmax = 256 nm (ε 25,300). The remaining 89 ml of basic stock solution was stirred for 0.5 h with 20 ml of Rexyn 101 cation exchange resin (pyridinium form) until the solution was pH=9.0 and the resin was filtered. The aqueous solution was evaporated to dryness and the residue applied to a silica gel plate. The plate was developed with MeOH : $CHCl_3$ (1:4) and the major band which ran identical to 9-(3-O-methyl-β-D-ribofuranosyl)adenine was removed. The mass spectrum of this material was identical to that of authentic 9-(3-O-methyl-β-D-ribofuranosyl)adenine.

A similar procedure on 41 and 44 gave 3' and 2'-O-methyl adenosine respectively.

9-(2-O-Methyl-3-O-p-nitrobenzenesulfonyl-β-D-ribofuranosyl)-adenine (41)

To a stirred solution of 1 g (0.00193 mole) of 33 (dried by evaporation using dry pyridine) in ~20 ml of dry pyridine was added 0.5 g (0.00226 mole) of p-nitrobenzenesulfonyl chloride with exclusion of moisture. After 5 days stirring at 24°, another 0.1 g (0.00045 mole) of p-nitrobenzenesulfonyl chloride was added. After stirring for a further 24 h, the reaction was poured into saturated aqueous $NaHCO_3$ (100 ml) and extracted with Et_2O (3 x 150 ml). The combined organic extract was evaporated to dryness. Coevaporation of toluene (2 x 60 ml) removed residual pyridine.

The resulting amorphous solid was dissolved in 80% aqueous HOAc (50 ml) and heated for 45 min at 100° in an oil bath. The solution was cooled, H_2O (50 ml) and EtOH (20 ml) was added, and the aqueous solution was extracted with n-pentane (4 x 100 ml) and evaporated to dryness. Coevaporation several times with toluene removed residual HOAc. The residue was dissolved in 98% EtOH and adsorbed on 10 g of silica gel. This powder was applied to the top of a dry-packed silica gel column (5 x 27 cm, 150 g). The column was eluted with MeOH : $CHCl_3$ (1:25). The first 260 ml of eluate was discarded and the following 350 ml was evaporated to dryness and crystallized from 98% EtOH (n-pentane, desiccator). The crystallization resulted in 0.35 g (39%) of 41.

This pure material had mp 185 - 185.5°; $[\alpha]_D^{24}$ -46.8° (c 1.4; DMF); uv (MeOH : H_2O [1:9]) max 255 nm (ε 25,500), min 224 nm (ε 8000); uv (MeOH : 0.1 *N* HCl [1:9]) max 255 nm (ε 25,700), min 223 nm (ε 6100); uv (0.1 *N* HCl) max 255 nm (ε 26,000), min 224 nm (ε 7500); nmr (DMSO-d_6) δ 3.11 (s, 3, OCH_3), 3.54 (m, 2, $H^{5'}$, $H^{5''}$), 4.13 (m, 1, $H^{4'}$), 4.76 (d of d, $J_{2'-1'}$ = 7.0 Hz, $J_{2'-3'}$ = 5.2 Hz, 1, $H^{2'}$), 5.49 (m, 2, $H^{3'}$, 5'-OH), 6.01 (d, $J_{1'-2'}$ = 7.0 Hz, 1, $H^{1'}$), 7.37 (br s, 2, 6-NH_2), 8.15, 8.36 (s, s; 1,1; H^2, H^8), 8.29 (d, *J meta*-*ortho* = 9.2 Hz, 2, aromatic protons), 8.50 (d, *J meta*-*ortho* = 9.2 Hz, 2, aromatic protons); ms (205°) m/e (some decomposition on probe (higher mass fragments)) 466 (0.25, M), 436 (0.15, e), 435 (0.10, M-OCH_3), 331 (0.40, w), 280 (3, M-$SO_2\phi NO_2$), 264 (2.5, M-$OSO_2\phi NO_2$), 263 (1.5, M-$HOSO_2\phi NO_2$), 245 (0.5), 234 (3, c-$OSO_2\phi NO_2$), 233 (4, c-$HOSO_2\phi NO_2$), 232 (2, M-(OCH_3 + $HOSO_2\phi NO_2$)), 174 (0.75), 173 (0.75, y-OCH_3), 164 (46, h), 148 (1.5, f), 136 (19, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{17}H_{18}N_6O_8S$: C, 43.77; H, 3.89; N, 18.02; S, 6.88. Found: C, 43.58; H, 3.91; N, 18:00; S 6.63.

9-(2-*O*-*p*-Aminobenzenesulfonyl-3-*O*-methyl-β-*D*-ribofuranosyl)adenine (42)

A solution of 0.25 g (0.00053 mole) of 40 in MeOH (40 ml), glacial HOAc (10 ml) and 5% Pd/C (0.05 g) was hydrogenated in a Paar shaker at 40 psi for 23 h. The reaction mixture was filtered through a small celite pad and then the celite pad was washed several times with MeOH. The solvent was evaporated from the filtrate and toluene and 98% EtOH were coevaporated several times to remove residual acid. A small amount of starting material remained so the above procedure was repeated exactly.

The residue was crystallized from acetone (*n*-pentane, desiccator) to give after two crops 0.211 g (90%) of *42*. Heavy spotting on TLC showed that this material contained a very small amount of starting material.

Recrystallization from acetone (*n*-pentane, desiccator) gave pure *42* which had mp 245 - 247°C; $[\alpha]_{D}^{24}$ - 45.6 (*c* 0.3, DMF); uv (MeOH : H_2O [1:9]) max 261 nm (ε 23,100), min 229 nm (ε 4500); uv (MeOH : 0.1 *N* HCl [1:9]) max 258 nm (ε 21,900), min 230 nm (ε 4300); uv (MeOH : 0.1 *N* NaOH [1:9]) max 261 nm (ε 23,300), min 229 nm (ε 4600); uv (0.1 *N* HCl) max 259.5 nm (ε 22,600), min 231 nm (ε 4000); nmr (DMSO-d_6) δ 3.31 (s, 3 (overlapped by H_2O peak), OCH_3), 3.60 (m, 2, $H^{5'}$, $H^{5''}$), 3.95 (d of d, $J_{3'-2'}$ = 5.0 Hz, $J_{3'-4'}$ = 2.6 Hz, 1, $H^{3'}$), 4.12 (m, 1, $H^{4'}$), 5.54 (d of d, $J_{2'-3'}$ = 5.0 Hz, $J_{2'-4'}$ = 7.0 Hz, 1, $H^{2'}$), 5.67 (br m, 1, 5'-OH), 6.08 (d, $J_{1'-2'}$ = 7.0 Hz, 1, $H^{1'}$), 6.18 (br s, 2, NH_2), 6.45 (d, *J* *meta*-*ortho* = 8.7 Hz, 2, aromatic *meta* protons), 7.25 (d, 2 (overlapped by NH_2), aromatic *ortho* protons), 7.29 (br s, 2 NH_2), 8.09, 8.20 (s,s; 1,1; H^2, H^8); ms (170°) m/e 436 (43, M), 406 (19.5, c), 405 (4, M-OCH_3), 375 (8, x), 333 (15.5, s + H), 308 (2), 302 (1.5, (s + H) - OCH_3), 280 (24, M-$SO_2\phi NH_2$), 264 (4, M $OSO_2\phi NH_2$), 255 (2.5), 240 (6.5), 234 (48, c-$OSO_2\phi NH_2$), 232 (6.5, M-(OCH_3 + $HOSO_2\phi NH_2$)), 205 (13), 204 (25, y), 202 (29), 194 (4), 190 (3.5, j), 177 (6, d-$SO_2\phi NH_2$), 173 (18, y-OCH_3 or/and $HOSO_2\phi NH_2$), 164 (57, h), 156 (100, $SO_2\phi NH_2$), 148 (9, f), 145 (7.5, s-(OCH_3 + $SO_2\phi NH_2$)), 140 (7.5, $SO\phi NH_2$), 136 (93, B + 2H), 135 (41, B + H).

Anal. Calcd for $C_{17}H_{20}N_6O_6S$: C, 46.78; H, 4.62; N, 19.26; S, 7.35. Found: C, 46.88; H, 4.65; N, 19.34; S, 7.37.

9-(3-*O*-*p*-Aminobenzenesulfonyl-2-*O*-methyl-β-*D*-ribofuranosyl)adenine (**43**)

A solution of 0.20 g (0.00042 mole) of **41** in MeOH (35 ml), glacial HOAc (10 ml and 5% Pd/C (0.05 g) was hydrogenated in a Paar shaker at 40 psi for 48 h. The reaction mixture was filtered through a small celite pad and the celite pad washed several times with MeOH. The filtrate was evaporated and toluene and EtOH were coevaporated several times to remove residual acid. An attempted crystallization from 98% ethanol resulted in discolouring of the product, so the material was applied to two silica gel plates. The plates were developed in MeOH : $CHCl_3$ [1:9] and the appropriate band eluted. The eluant was evaporated to dryness and crystallized from acetone (*n*-pentane, desiccator) to give 0.027 g of slightly contaminated **43**. The mother liquor contained pure product and was precipitated from acetone and pentane to give 0.104 g (57%) of pure *43*.

This chromatographically pure precipitate had mp 115 - 125°; $[\alpha]_{D}^{24}$ -50.1 (*c* 0.56, DMF); uv (MeOH : H_2O [1:9]) max 266 nm (ε 27,400), min 228 nm (ε 3700); uv (MeOH : 0.1 *N* HCl [1:9])max 264 nm (ε 22,000), min 231 nm (ε 4000); uv (MeOH : 0.1 *N* NaOH [1:9]) max 266 nm (ε 27,900), min 228 nm (ε 2700); uv (0.1 *N* HCl) max 263 nm (ε 22,900), min 231.5 nm (ε 4800); nmr (DMSO-d_6) δ 3.12 (s, 3, OCH_3), 3.48 (br d of d, $J_{5'-5''}$ ≈ 13.0 Hz (overlapped by H_2O), 2, $H^{5'}$, $H^{5''}$), 4.06 (m, 1, $H^{4'}$), 4.62 (d of d, $J_{2'-3'}$ = 5.4 Hz, $J_{2'-1'}$ = 7.6 Hz, 1, $H^{2'}$), 5.12 (d of d, $J_{3'-2'}$ = 5.4 Hz, $J_{3'-4'}$ = 1.8 Hz, 1, $H^{3'}$), 5.62 (br s, 1, 5' -OH), 5.98 (d, $J_{1'-2'}$ = 7.6 Hz, 1, $H^{1'}$), 6.31 (br s, 2, NH_2), 6.70 (d, *J* *meta*-*ortho* = 8.8 Hz, 2, aromatic *meta* protons), 7.38 (br s, 2, NH_2), 7.60 (d, *J* *ortho*-*meta* = 8.8 Hz, 2, aromatic *ortho* protons), 8.14, 8.36 (s, s; 1,1; H^2, H^8); ms (170°) m/e 436 (14, M), 280 (33, M-SO_2ϕNH_2), 264 (10, M-SO_2ϕNH_2), 264 (10, M-OSO_2ϕNH_2), 234 (45, x), 233 (9.5, M-(OCH_3 + OSO_2ϕNH_2)), 232 (4, M-(OCH_3 + $HOSO_2$ϕNH_2)) 223 (1.5), 204 (1.5, y), 202 (8), 192 (10, d), 191 (1.5), 190 (1, j), 179 (1.5), 177 (2, v), 175 (2), 173 (30, y-OCH_3 and/or $HOSO_2$ϕNH_2), 164 (35, h), 156 (31, SO_2ϕNH_2), 149 (35, e), 148 (6, f), 145 (3.5, s-(OCH_3 + SO_2ϕNH_2)), 140 (5, SOϕNH_2), 135 (100, B + 2H), 135 (92, B + H).

Anal. Calcd for $C_{17}H_{20}N_6O_6S$: C, 46.78; H, 4.62; N, 19.26; S, 7.35. Found: C, 46.74; H, 4.59; N, 19.10; S, 7.28.

9-(3-_O_-Methyl-2-_O_-trifluoromethylsulfonyl-β-_D_-ribofuranosyl)-adenine (44).

To a stirred solution of 0.5 g (0.00096 mole) of 18 (dried by repeated evaporation using dry pyridine) in ~50 ml of dry pyridine (under a flow of dry nitrogen and protected by a drying tube) in a dry-ice and acetone bath at -35°, was injected 0.35 ml (~0.0024 mole) of trifluoromethylsulfonyl anhydride dropwise (over ~5 min). A precipitate formed on addition of the anhydride and the solution turned yellow. The mixture was stirred for 6 h at <-20° and was then poured into a stirred mixture of Et_2O (50 ml) and saturated aqueous $NaHCO_3$ (50 ml). The Et_2O layer was separated and the aqueous layer extracted again with Et_2O (20 ml). The combined organic extracts were evaporated to dryness and coevaporated using toluene, 98% EtOH and finally Et_2O several times.

The yellow residue was dissolved in 80% aqueous HOAc (30 ml) and heated at 100° for 15 min. The now pale yellow solution was cooled and diluted with H_2O (30 ml). The resulting solution was extracted with _n_-pentane (4 x 100 ml) and evaporated to dryness. Again, coevaporation with toluene, H_2O and Et_2O several times effected removal of residual acid. The residue was stirred with MeOAc (50 ml), Et_2O (50 ml) and H_2O (50 ml) and the upper layer was separated and evaporated to dryness. This residue was dissolved in a small amount of $CHCl_3$ and applied to a silica gel column (2.5 x 42 cm, 50 g) packed in $CHCl_3$. The column was eluted with MeOAc. After the first 300 ml of eluate was discarded, the next 200 ml was collected and evaporated to dryness to give 0.14 g (34%) of crude *44*. This material was crystallized from Et_2O (*n*-pentane, desiccator) to give in two crops 0.09 g (22%) of pure crystalline *44*.

This material had mp 145 - 148° (with decomposition); $[\alpha]_{\underline{D}}^{24}$ -40.6 (*c* 0.47, DMF); uv (MeOH : H_2O [1:9]) max 257 nm (ε 14,800), min 226 nm (ε 1900); uv (0.1 *N* HCl) max 256 nm (ε 15,000), min 226 nm (ε 2600); nmr (DMSO-$\underline{d}_6$) δ 3.47 (s, 3, OCH_3), 3.72 (m, 2, $H^{5'}$, $H^{5''}$), 4.20 (m, 1, $H^{4'}$), 4.39 ("t", $\underline{J}_{3'-2'} \approx \underline{J}_{3'-4'} \approx 5.0$ Hz, 1, $H^{3'}$), 5.57 (br s, 1, 5' - OH), 6.11 ("t", $\underline{J}_{2'-1'} \approx \underline{J}_{2'-3'} \approx 4.6$ Hz, 1, $H^{2'}$), 6.41 (d, $\underline{J}_{1'-2'} = 4.5$ Hz, 1, $H^{1'}$), 7.42 (br s, 2, 6-NH_2), 8.18, 8.40 (s,s; 1,1; H^2, H^8); ms (125°) m/e 413 (5, M), 396 (0.12, M-OH), 383 (10.5, c), 382 (1.5, M-OCH_3), 368 (2.5, *c*-CH_3), 344 (1, M-CF_3), 310 (2,d), 280 (1, M-SO_2CF_3), 264 (3.5, M-OSO_2CF_3), 263 (3, M-$HOSO_2CF_3$), 262 (1.5, M-(CH_2O + SO_2CF_3)), 235 (3.5), 234 (5.5, *c*-OSO_2CF_3), 233 (2, M-(OCH_3 + OSO_2CF_3)), 232 (2, M-(OCH_3 + $HOSO_2CF_3$)), 204 (44, y), 202 (17), 194 (2), 190 (3.5,j), 189 (3), 177 (5, d-SO_2CF_3), 173 (4.5, y-OCH_3), 164 (34, h), 148 (5.5, f), 136 (64, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{12}H_{14}N_5O_6SF_3$: C, 34.87; H, 3.41; N, 16.82; S, 7.76; F, 13.79. Found: C, 34.83; H, 3.51; N, 16.82; S, 8.03; F, 13.96.

9-(2-*O*-Methyl-3-*O*-trifluoromethylsulfonyl-β-*D*-ribofuranosyl)adenine (45).

To a vigorously stirred solution of 1.09 (0.00192 mole) of 37 (dried by repeated evaporation using dry pyridine) in ~50 ml of dry pyridine, in a dry-ice, acetone bath at ≤-25°, was added 1.0 ml (0.0069 mole) of trifluoromethylsulfonyl anhydride in 10 ml pyridine dropwise over 15 min with exclusion of moisture. The resulting reddish yellow solution was stirred for 2.5 h at ~-25° and poured into a stirred mixture of saturated aqueous $NaHCO_3$ (125 ml) and Et_2O (100 ml). The organic layer was separated and the aqueous layer diluted with 100 ml of H_2O and extracted again with 100 ml of Et_2O. The combined yellow organic extracts were washed with H_2O (100 ml), evaporated to dryness, and coevaporated using toluene and CH_2Cl_2 several times to remove residual pyridine.

The residue was dissolved in 90% aqueous trifluoroacetic acid (15 ml) giving a reddish yellow solution. To this solution was added H_2O (75 ml) and the solution turned pink in colour and a precipitate appeared. The pink solution was extracted with *n*-pentane (100 ml) and CH_2Cl_2 (3 x 50 ml). The combined CH_2Cl_2 extracts were washed with saturated, aqueous $NaHCO_3$ (75 ml). The original aqueous solution was adjusted to pH 9 by addition of $NaHCO_3$, diluted with 100 ml of H_2O and extracted with CH_2Cl_2 (2 x 75 ml). All the CH_2Cl_2 layers were combined, washed with H_2O (50 ml) and evaporated to dryness. The residue was dissolved in a small amount of $CHCl_3$ and applied to an alumina column (1.8 x 37 cm, 70 g) packed in $CHCl_3$ and eluted with MeOH : $CHCl_3$ (1:24). After the first 180 ml of eluate was discarded, the next 90 ml of eluate was concentrated and the product precipitated by dropwise addition to *n*-pentane to give 0.127 g (16%) of pure **45**. This material was quite unstable and decomposed slightly over a period of weeks even when stored over $CaSO_4$ at 2°.

The pure material had uv (0.1 *N* HCl) max 256.5 nm (ε 15,000) min 229 nm (ε 4100); nmr (DMSO-d_6) δ 3.34 (s, 3, OCH_3), 3.68 (m, 2, $H^{5'}$, $H^{5''}$), 4.42 (br "t", $J_{4'-5'} \simeq J_{4'-5''} \simeq 4.5$ Hz, 1, $H^{4'}$), 5.06 (d of d, $J_{2'-3'} = 4.7$ Hz, $J_{2'-1'} = 7.8$ Hz, 1, $H^{2'}$), 5.38 (br s, 1, 5'-OH), 5.84 (br "d", $J_{3'-2'} = 4.7$ Hz, 1, $H^{3'}$), 6.07 (d, $J_{1'-2'} = 7.8$ Hz, 1, $H^{1'}$) 7.81 (br s, 2, 6-NH_2), 8.24, 8.39 (s,s;1,1; H^2,H^8); ms (140°) m/e 413 (20, M), 383 (0.7, c), 344 (5.5, M-CF_3), 280 (s, M-SO_2CF_3), 264 (10.5, M-OSO_2CF_3), 263 (6, M-$HOSO_2CF_3$), 248 (2.5 s-OCH_3), 234 (20, x), 233 (19, M-)OCH_3 + OSO_2CF_3)), 232 (5, M-(OCH_3 + $HOSO_2CF_3$)), 225 (3.5), 204 (2.5, y), 202 (11), 201 (5.5), 192 (4.5, d), 177 (1, v), 173 (2, y-OCH_3), 164 (4, h), 149 (6.5,e), 148 (3, f), 136 (28, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{12}H_{14}N_5O_6SF_3$: C, 34.87; H, 3.41; N, 16.94; S, 7.76. Found: C, 35.09; H, 3.41; N, 16.84; S, 7.46.

Mass spectra of biproducts of hydrolysis of 44 and 45.

Biproduct of 44 hydrolysis gave ms (240°) m/e 299 (4, M), 269 (3.5, c), 268 (8, M-OCH_3), 264 (4, M-Cl), 249 (2.5, M-(Cl + CH_3)), 238, (4.5, x), 234 (7, c-Cl), 232 (3.5, M-(OCH_3 + HCl)), 213 (3, i + H), 204 (7, y), 202 (5.5), 196 (3.5, d), 173 (5, y-OCH_3), 164 (29, h), 149 (11, e), 142 (8), 136 (94, B + 2H), 135 (100, B + H).

Biproduct of 45 hydrolysis gave ms (150°) m/e 299 (11, M), 269 (3, c), 268 (2, M-OCH_3), 264 (34, M-Cl), 256 (8), 234 (55, x), 232 (4, M-OCH_3 + HCl)), 202 (10), 192 (5,d), 173 (6, y-OCH_3), 164 (73, h), 163 (9), 149 (19, e), 136 (94, B + 2H), 135 (100, B + H).

9-(2-O-Benzylsulfonyl-3-O-methyl-β-D-ribofuranosyl)adenine (36).

To a stirred solution of 0.3 g (0.00058 mole) of 18 (dried by repeated evaporation using dry pyridine) in ~20 ml of dry pyridine was added 0.12 g (0.00063 mole) of benzylsulfonyl chloride. This solution was stirred for 0.5 h with exclusion of moisture and then was poured into a stirred solution of saturated aqueous $NaHCO_3$ (100 ml) and CH_2Cl_2 (100 ml). The CH_2Cl_2 was separated, washed with H_2O (50 ml), evaporated to dryness and the residue was coevaporated several times with toluene and CH_2Cl_2 to remove residual solvent.

The resulting amorphous solid was dissolved in 80% aqueous HOAc (25 ml) and heated to reflux for 25 min. The reaction mixture was cooled, H_2O added (25 ml) and the aqueous solution extracted with *n*-pentane (3 x 75 ml). The aqueous solution was evaporated to dryness and toluene was added and coevaporated several times to remove residual acid. The two reaction sequence proceeded quantitatively with no other products or starting material observable by TLC. The residue was "crystallized" from EtOH, H_2O to give 0.186 g (75%) of 36 in two crops.

The purified material had mp 135-145° (slowly softens between these limits); $[\alpha]_D^{24}$ -42.1° (*c* 0.78, DMF); uv (MeOH) max 260 nm (ε 14,000), min 228 nm (ε 2700); uv (MeOH : 0.1 *N* HCl [1:9]) max 257.5 nm (ε 13,900), min 229.5 nm (ε 3200); uv (MeOH : 0.1 *N* NaOH) max 260 nm (ε 13,700), min 228 nm (ε 1900); nmr (DMSO-d_6) δ 3.42 (s, 3, OCH_3), 3.68 (m, 2, $H^{5'}$,$H^{5''}$), 4.19 (m, 2, $H^{4'}$, $H^{3'}$), 4.76 (s, 2, CH_2), 5.54 (br s, 1, 5'-OH), 5.72 ("t", $J_{2'-1'} \approx J_{2'-3'} \approx 4.8$ Hz, 1, $H^{2'}$), 6.21 (d, $J_{1'-2'} = 5.3$ Hz, 1, $H^{1'}$), 7.25 (s, 5, aromatic protons), 7.40 (br s, 2, 6-NH_2), 8.17, 8.36 (s,s; 1,1; H^2,H^8); ms (160°) m/e 435 (6.5, M), 405 (23, c), 404 (2.5, M-OCH_3), 374 (4.5, x), 332 (13, d), 280 (16, M-$SO_2CH_2\phi$), 268 (5), 264 (19, M-$OSO_2CH_2\phi$), 262 (2.5), 234 (45, c-$OSO_2CH_2\phi$), 232 (10, M-(OCH_3 + $HOSO_2CH_2\phi$)), 226 (4), 224 (10), 220 (2.5), 218 (1.5), 204 (39, y), 202 (32), 194 (3), 190 (2,j), 178 (3), 177 (5.5, d-$SO_2CH_2\phi$), 173 (3.5, y-OCH_3), 164 (60, h), 148 (13.5, f), 145 (2.5), 136 (100, B + 2H), 135 (71, B + H).

Anal. Calcd for $C_{18}H_{21}N_5O_6S$: C, 49.64; H, 4.86; N, 16.08; S, 7.36. Found: C, 49.81; H, 5.04; N, 16.30; S, 7.44.

9-(3-O-Benzylsulfonyl-2-O-methyl-β-D-ribofuranosyl)adenine (37).

To a stirred solution of 0.3 g (0.00058 mole) of 33 (dried by repeated evaporation using dry pyridine) in ~20 ml of dry pyridine was added 0.12 g (0.00063 mole) of benzylsulfonyl chloride. This solution was stirred for 20 min with exclusion of moisture and then was poured into saturated aqueous $NaHCO_3$ (75 ml). This aqueous solution was extracted with CH_2Cl_2 (75 ml) and the organic extract was washed with H_2O (75 ml). The resulting solution was evporated to dryness and toluene coevaporated several times to remove residual pyridine.

The resulting amorphous solid was dissolved in hot aqueous 80% HOAc (25 ml) and heated at 100° for 15 min in an oil bath. The acid solution was cooled and H_2O (25 ml) was added. The resulting aqueous solution was extracted with n-pentane (3 × 50 ml) and evaporated to dryness. Toluene was coevaporated to remove residual acid. Again, both reactions proceeded quantitatively (except for a trace amount of remaining starting material) by TLC. The residue was dissolved in CH_2Cl_2 (75 ml) and washed with H_2O (2 x 50 ml). The CH_2Cl_2 was dried with anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in ~1 ml of hot 95% EtOH cooled slightly and 60 ml of Et_2O added rapidly while scratching the flask. Crystals rapidly formed and gave 0.177 g (70%) of pure *37*.

This material had mp 99-101°; $[\alpha]_{D}^{24}$ -68.5° (*c* 0.59, DMF); uv (MeOH) max 260 nm (ε 15,300) min 228 nm (ε 3500); uv (MeOH : 0.1 *N* HCl [1.9]) max 257.5 nm (ε 15,500), min 229.5 nm (ε 3800); uv (MeOH: 0.1 *N* NaOH [1.9]) max 259.5 nm (ε 15,600), min 226 nm (ε 2200); nmr (DMSO-d_6) δ 3.33 (s, 3, OCH_3), 3.63 (m, 2, $H^{5'}$,$H^{5''}$), 4.26 (m, 1, $H^{4'}$), 4.76 (d of d (overlapped by CH_2), $J_{2'-1'}$ = 7.3 Hz, $J_{2'-3'}$ = 5.1 Hz, 1, $H^{2'}$), 4.83 (br s, 2, CH_2), 5.37 (br d, $J_{3'-2'}$ ≃ 5.0 Hz, 1, $H^{3'}$), 5.72 (br "t", $J_{OH-5'}$ ≃ $J_{OH-5''}$ ≃ 5.0 Hz, 1, 5'-OH), 6.02 (d, $J_{1'-2'}$ = 7.3 Hz, 1, $H^{1'}$), 7.42 (m, 7, aromatic protons, 6-NH_2), 8.17, 8.41 (s,s; 1,1; H^2,H^8), 3.29 (s, 0.5 (rel. int.), H_2O); ms (190°) m/e 435 (1.5, M), 405 (1.5, c), 300 (3, w), 280 (38, M-$SO_2CH_2\phi$), 269 (1), 264 (27, M-$OSO_2CH_2\phi$), 263 (2, M-$HOSO_2CH_2\phi$), 250 (0.9, c-$SO_2CH_2\phi$), 240 (1.5), 234 (66, x) 233 (14, M-(OCH_3 + $OSO_2CH_2\phi$)), 232 (4.5, M-(OCH_3 + $HOSO_2CH_2\phi$)), 226 (1.5), 224 (1.5), 218 (1.5), 204 (2.5,y), 202 (21), 192 (16,d), 189 (3.5), 177 (3,v), 176 (3), 173 (3.5,y-OCH_3), 164 (52, h), 148 (12.5,f), 146 (11, s-$SO_2CH_2\phi$), 136 (100, B + 2H), 135 (98, B + H).

Anal.Calcd for $C_{18}H_{21}N_5O_6S \cdot 0.5\ H_2O$: C, 48.64; H, 4.99; N, 15.76; S, 7.22. Found: C, 48.50; H, 4.76; N, 15.53; S, 7.41.

Preparation of 9-(3-*O*-Benzyl-β-*D*-ribofuranosyl)adenine (46) and 9-(2-*O*-Benzyl-β-*D*-ribofuranosyl)adenine (47)

These compounds were prepared from adenosine by the method of Christensen and Broom [151] using phenyldiazomethane and stannous chloride.

Compound 46 was crystallized from acetone to give a material which had mp 198.5 - 199.5°; uv (0.1 *N* HCl) max 256 nm (ε 14,900) min 229 nm (ε 3900); nmr (DMSO-d_6) δ 3.64 (m, 2, $H^{5'}$,$H^{5''}$), 4.14 (m, 2, $H^{3'}$,$H^{4'}$), 4.62 (d,(half AB quartet), J_{CH_2} geminal = 12.0 Hz, 1, half benzyl CH_2), 4.79 (d (other half AB quartet), J_{CH_2} geminal = 12.0 Hz, 1, half benzyl CH_2), 4.85 (m, 1, $H^{2'}$), 5.48 (d of d, $J_{OH-5'}$= 4.6 Hz, $J_{OH-5''}$ = 7.0 Hz, 1, 5'-OH), 5.57 (d, $H_{OH-2'}$ = 6.4 Hz, 1, 2'-OH), 5.95 (d, $J_{1'-2'}$ = 6.6 Hz, 1, $H^{1'}$), 7.34 (m, 7, aromatic protons, 6-NH_2), 8.14, 8.36 (s,s;1,1;H^2,H^8); ms (175°) m/e 351 (1.5, M), 327 (8,c), 326 (1, M-CH_2OH), 266 (47, M-$CH_2\phi$), 251 (17, M-OCHφ), 250 (1.5, M-$OCH_2\phi$), 234 (5, M-(OCHφ + OH)), 226 (2.5), 224 (2.5), 220 (21,x), 202 (5), 178 (23, d), 164 (73, h), 148 (12, f), 136 (100, B + 2H), 135 (72, B + H).

Anal.Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.42; H, 5.62; N, 19.45.

The compound 47 was crystallized from acetone to give a material which had mp 192-195°; uv (0.1 N HCl) max 258 nm (ε 12,100), min 232 nm (ε 4000); nmr (DMSO-d_6) δ 3.64 (m, 2, $H^{5'}$, $H^{5''}$), 4.03 (m, 1, $H^{4'}$), 4.38 (m, 1, $H^{3'}$), 4.44 (d (half of AB quartet), J_{CH_2} geminal = 12.0 Hz, 1, half benzyl CH_2), 4.55 (m (overlapped by AB quartet), 1, $H^{2'}$), 4.67 (d (half of AB quartet), J_{CH_2} geminal = 12.0 Hz, 1, half benzyl CH_2), 5.36 (m, 2, 3'-OH, 5'-OH), 6.07 (d, $J_{1'-2'}$ = 6.4 Hz, 1, $H^{1'}$), 7.17 (s, 5, aromatic protons), 7.30 (br s, 2, 6-NH_2), 8.09, 8.31 (s,s;1,1;H^2, H^8); ms (175°) m/e 357 (1, M), 327 (6.5, c), 310 (0.7, x), 268 (28, d), 266 (5.5 M-CH_2ϕ), 251 (28, M-OCHϕ), 234 (2.5 M-(OCHϕ + OH)), 226 (3), 224 (4.5), 222 (1.5, w), 221 (2.5, c-OCHϕ), 220 (1.5), 202 (1.5), 192 (1), 190 (2, j), 177 (7.5, d-CH_2ϕ), 164 (13.5, h), 162 (2.5), 148 (8, f), 136 (100, B + 2H), 135 (96, B + H).

Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 56.94; H, 5.34; N, 19.40.

9-(2-O-p-Methoxyphenylacetyl-3-O-methyl-β-D-ribofuranosyl) adenine (48).

To a stirred solution of 0.285 g (0.0017 mole) of p-methoxyphenylacetic acid in 5 ml of dry EtOAc, was added 0.45 g (0.0022 mole) of N,N'-dicyclohexylcarbodiimide. This solution was stirred with exclusion of moisture at 24° for 1 h giving a white precipitate. The solution was filtered into a stirred solution containing 0.3 g (0.00058 mole) of 18 in dry EtOAc (5 ml) and CH_2Cl_2 (5 ml). The filter flask was kept at 15° in an ice bath with exclusion of mixture. No 2'-O-acylation had occurred after 5 min (TLC) and so 0.050 g (0.0004 mole) of 4-N,N-dimethylaminopyridine was added. After a further 5 min the reaction was complete (TLC). The mixture was filtered and the dicyclohexyl urea filter cake was washed with more EtOAc. The resulting clear organic solution was washed with saturated aqueous $NaHCO_3$ (30 ml), H_2O (2 x 30 ml) and evaporated to dryness. Acetone was added and coevaporated several times to remove residual H_2O and CH_2Cl_2 was added. The CH_2Cl_2 solution was evaporated to a volume of ~2 ml and the product was precipitated by addition to n-pentane (100 ml).

The precipitate was filtered and dissolved in 30 ml of hot 80% aqueous HOAc. The resulting solution was stirred for 15 min at 100° in an oil bath. The solution was cooled, 30 ml of H_2O added and the resulting aqueous solution extracted with n-pentane (3 x 50 ml). The aqueous solution was evaporated to dryness. Toluene and 98% EtOH were added and coevaporated several times to remove residual acid.

The residue was crystallized from EtOH and H_2O to give 0.20 g (81%) of pure 48 in two crops.

This material had mp 159-163°; $[\alpha]_D^{24}$ -48.0 (c 0.68 DMF); uv (MeOH : H_2O [1:9]) max 260.5 nm (ε 14,500), min

236 nm (ε 8300) sh ~227 nm (ε 9000); nmr (DMSO-d_6) δ 3.31 (s, 3, OCH_3), 3.63 (br s, 4, $H^{5'}$, $H^{5''}$, CH_2), 3.71 (s, 3, OCH_3), 4.17 (m, 2, $H^{4'}$, $H^{3'}$), 5.49 (br s, 1, 5'-OH), 5.78 ("t", $J_{2'-1'} \simeq J_{2'-3'} \simeq$ 5.2 Hz, 1, $H^{2'}$), 6.17 (d, $J_{1'-2'}$ = 5.6 Hz, 1, $H^{1'}$), 6.79 (d, *J* *ortho*-*meta* = 8.8 Hz, aromatic protons), 7.10 (d, *J* *ortho*-*meta* = 8.8 Hz, aromatic protons), 7.36 (br s, 2, 6-NH_2), 8.15, 8.36 (s,s; 1,1; H^2,H^8); ms (215°) m/e 429 (49, M), 399 (1.5, c), 398 (0.5, M-OCH_3), 368 (2.5, x), 326 (6.5, d), 294 (20, w), 280 (5, M-$COCH_2OCH_3$), 266 (1.5), 251 (1), 234 (8, c-$OCOCH_2\phi OCH_3$), 233 (1.5, M-(OCH_3 + $OCOCH_2\phi OCH_3$)), 232 (2, M-(OCH_3 + $HOCOCH_2\phi OCH_3$)), 220 (2), 204 (3.5, y), 202 (6.5), 190 (1,j), 178 (6.5), 177 (2, d-$COCH_2\phi OCH_3$), 173 (2, y-OCH_3), 166 (1.5, $HOCOCH_2OCH_3$), 164 (13, h), 148 (100, f), 136 (28, B + 2H), 135 (14.5, B + H).

Anal. Calcd for $C_{20}H_{23}N_5O_6$: C, 55.94; H, 5.40; N, 16.31. Found: C, 55.91; H, 5.49; N, 16.17.

9-(2-*O*-*p*-Methoxybenzoyl-3-*O*-methyl-β-*D*-ribofuranosyl)-adenine (50).

To a stirred solution of 0.262 g (0.0017 mole) of *p*-methoxybenzoic acid, in 10 ml of dry EtOAc, was added 0.369 (0.00175 mole) of *N*,*N*'-dicyclohexylcarbodiimide. This solution was stirred with exclusion of moisture at 24° for 1.5 h and then filtered into a stirred solution containing 0.3 g (0.00058 mole) of 18 in dry EtOAc (20 ml) and CH_2Cl_2 (2 ml) and 0.05 g (0.0004 mole) of 4-*N*,*N*'-dimethylaminopyridine. This solution was stirred for 1.5 days and another 0.1 g (0.0008 mole) of dimethylaminopyridine was added. After stirring for another 3.5 days, the reaction was poured into 75 ml of saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (75 ml). The CH_2Cl_2 layer was washed with H_2O (50 ml) containing some salt and evaporated to dryness. This residue was dissolved in a small volume (~5 ml) of CH_2Cl_2, filtered and then precipitated by addition to *n*-pentane (100 ml).

The precipitate was dissolved in 25 ml of hot (aqueous) 80% HOAc. This solution was heated at 100° for 15 min in an oil bath. The solution was cooled and 30 ml of H_2O added. The aqueous layer was extracted with *n*-pentane (3 x 75 ml) and evaporated to dryness. Toluene was added and coevaporated to remove residual acid. The residue was crystallized from EtOH and H_2O to give 0.11 g (47%) of pure **50**. The mother liquor was purified by application to a silica gel column and elution with MeOAc to give a further 0.033 g. Total yield of **50**, 0.143 g (67%).

This material had mp 195-196°; $[\alpha]_D^{24}$ - 153.7° (*c* 0.56, DMF); uv (MeOH : H_2O [1:9]) max 262 nm (ε 30,000), min 227.5 nm (ε 3700); nmr (DMSO-d_6) δ 3.36 (s, 3, OCH_3), 3.75 (m, 2, $H^{5'}$, $H^{5''}$), 3.83 (s, 3, OCH_3), 4.24 (m, 1, $H^{4'}$), 4.36 ("t", $J_{3'-2'} \approx J_{3'-4'} \approx$ 5.6 Hz, 1, $H^{3'}$), 5.55 (br "t", $J_{OH-5'} \approx J_{OH-5''} \approx$ 5.0 Hz, 1, 5'-OH), 5.97 ("t", $J_{2'-1'}$ $J_{2'-3'}$ 5.0 Hz, 1, $H^{2'}$), 6.34 (d, $J_{1'-2'}$ = 5.5 Hz, 1, $H^{1'}$), 7.07 (d, *J* *ortho*-*meta* - 9.0 Hz, 2, aromatic protons), 7.37 (br s, 2, 6-NH$_2$), 7.95 (d, *J* *ortho*-*meta* = 9.0 Hz, 2, aromatic protons), 8.20, 8.46 (s, s; 1,1; H^2, H^8); ms (200°) m/e 415 (0.2, M), 385 (0.75, c), 384 (0.1, M-OCH$_3$), 354 (0.2, x), 312 (1, d), 280 (5.5, M-COϕOCH$_3$ and/or w), 270 (0.4), 264 (1, M-OCOϕOCH$_3$), 234 (0.4, c-OCOϕOCH$_3$), 233 (0.3 M-(OCH$_3$ + OCOϕOCH$_3$)), 232 (0.55, M-(OCH$_3$ + HOCOϕOCH$_3$)), 220 (0.25), 204 (4, y), 202 (2.5), 190 (0.4, j), 177 (0.7, d-COϕOCH$_3$), 173 (0.8, y-OCH$_3$), 164 (4,h), 162 (6.4), 152 (2.5, HOCOϕOCH$_3$), 148 (2.5, f), 145 (0.5, w-COϕOCH$_3$), 136 (20, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{19}H_{21}N_5O_6$: C, 54.93; H, 5.10; N, 16.86. Found: C, 54.82; H, 5.17; N, 16.82.

9-(3-*O*-*p*-Methoxyphenylacetyl-2-*O*-methyl-β-*D*-ribofuranosyladenine (*49*).

To a stirred solution of 0.38 g (0.0023 mole) of *p*-methoxyphenylacidic acid in 5 ml of dry EtOAc was added 0.6 g (0.0029 mole) of *N*,*N*'-dicyclohexylcarbodiimide with exclusion of moisture. This solution was stirred at 24° for 1 h giving a white precipitate. The mixture was filtered into a stirred solution containing 0.3 g (0.00058 mole) of *33* and 0.05 g (0.0004 mole) of 4-*N*,*N*'-dimethylaminopyridine in 5 ml of dry EtOAc. The reaction was stirred for 5 min and filtered. The dicyclohexylurea precipitate was washed with saturated aqueous NaHCO$_3$ (30 ml) and H$_2$O (2 x 30 ml). The organic layer was then evaporated to dryness and acetone was added and coevaporated several times to remove residual H_2O. The residue was taken up in ~2 ml CH_2Cl_2 and precipitated by addition to *n*-pentane (100 ml).

The precipitate was filtered and dissolved in 30 ml of hot aqueous 80% HOAc. This solution was heated for 15 min at 100° in an oil bath. The reaction was cooled and 30 ml of H_2O added. The aqueous solution was extracted with *n*-pentane (3 x 75 ml) and evaporated to dryness. Toluene was added and coevaporated several times to remove residual acid. The residue, in a small amount of CH_2Cl_2, was applied to an alumina column (1.5 x 27 cm, 30 g) wet packed in CH_2Cl_2. The column was eluted with MeOH : CH_2Cl_2 (3:97). After the first 250 ml of eluate was discarded, the next 250 ml was evaporated to dryness to give 0.2 g (80%) of pure **49**. This residue was "crystallized" from Et_2O to give in two crops 0.122 g (49%) of **49**.

This material had mp 152 - 156°; $[\alpha]_{D}^{24}$ - 41.4° (*c* 0.68,DMF); uv (MeOH : H_2O [1:9]) max 260 nm (ε 15,200), min 237 nm (ε 7400) sh ~227 nm (ε 9500); nmr (DMSO-d_6) δ 3.19 (s, 3, OCH_3), 366 (m, 2, $H^{5'}$, $H^{5''}$), 3.73 (s, 5, OCH_3, CH_2), 4.16 (m, 1, $H^{4'}$), 4.70 (d of d, $J_{2'-1'}$ = 7.3 Hz, $J_{2'-3'}$ = 5.2 Hz, 1, $H^{2'}$), 5.49 (d of d, $J_{3'-2'}$ = 5.2 Hz, $J_{3'-4'}$ = 1.8 Hz, 1, $H^{3'}$) 5.61 (br s, 1, 5'-OH), 6.01 (d, $J_{1'-2'}$ = 7.3 Hz, 1, $H^{1'}$), 6.89 (d, *J* *meta*-*ortho* = 8.6 Hz, 2, aromatic protons), 7.24 (d, *J* *meta*-*ortho* = 8.6 Hz, 2, aromatic protons), 7.37 (br s, 2, 6-NH_2), 8.16, 8.41 (s, s; 1,1; H^2, H^8); ms (240°) m/e 429 (6, M), 399 (5,c), 398 (2, M-OCH_3), 295 (18, s), 294 (6, w), 284 (1.5), 280 (7, M-$COCH_2\phi OCH_3$), 264 (6.5, M-$OCOCH_2\phi OCH_3$), 234 (66, x), 232 (1, M-(OCH_3 + $HOCOCH_2\phi OCH_3$)), 204 (1, y), 202 (16), 192 (19, d), 190 (1, j), 177 (1.5, v), 176 (1.5), 173 (3.5, y-OCH_3), 166 (3, $HOCOCH_2\phi OCH_3$), 164 (33, h), 162 (1.5), 149 (6.5, e), 148 (52, f), 146 (3.5,s-$COCH_2\phi OCH_3$), 136 (100, B + 2H), 135 (54, B + H).

Anal. Calcd for $C_{20}H_{23}N_5O_6$: C, 55.94; H, 5.40; N, 16.31. Found: C, 56.08; H, 5.70; N, 16.46.

9-(3-*O*-*p*-Methoxybenzoyl-2-*O*-methyl-β-*D*-ribofuranosyl)adenine (*51*).

To a stirred solution of 0.35 g (0.0024 mole) of *p*-methoxybenzoic acid in 10 ml of dry EtOAc was added 0.48 g (0.0024 mole) of *N*,*N*'-dicyclohexylcarbodiimide with exclusion of moisture. This solution was stirred at 24° for 1 h and filtered into a stirred solution containing 0.3 g (0.00058 mole) of 4-*N*,*N*-dimethylaminopyridine. This solution was stirred for 7 days and filtered. The filter cake was washed with EtOAc and the combined EtOAc solution was washed with saturated aqueous $NaHCO_3$ (50 ml) and H_2O containing some salt (2 x 50 ml). The EtOAc solution was evaporated to dryness, dissolved in a small amount of CH_2Cl_2 (~5 ml) and precipitated by addition to *n*-pentane (100 ml).

The precipitate was dissolved in 25 ml of hot, aqueous 80% HOAc and heated for 15 min at 100° in an oil bath. The reaction was then cooled and 25 ml of H_2O added. The aqueous solution was extracted with *n*-pentane (3 x 50 ml) and evaporated to dryness. Toluene was added and coevaporated several times to remove residual acid. The residue was dissolved in a small amount of MeOAc and applied to a silica gel column (2.6 x 20 cm, 30 g) packed in MeOAc. The column was eluted with MeOAc. After the first 280 ml of eluate was discarded, the next 330 ml was collected and evaporated to dryness to give 0.123 g (58%) of crude *51*. This crude material, containing small amounts of impurities, was applied to a silica gel plate, developed three times in MeOAc and the appropriate band eluted. The residue was crystallized from EtOH and H_2O to give 0.07 g (33%) of pure *51*.

This material had mp 199 - 200°; $[\alpha]_D^{24}$ - 145.0° (*c* 0.33, DMF); uv (MeOH : H_2O [1:9]) max 261 nm (ϵ 28,900), min 227.5 nm (ϵ 4000); nmr (DMSO-d_6) δ 3.24 (s, 3, OCH_3), 3.33 (br s, 1, 5'-OH), 3.76 (m, 2, $H^{5'}$, $H^{5''}$), 3.85 (s, 3, OCH_3), 4.33 (m, 1, $H^{4'}$), 4.81 (d of d, $J_{2'-1'}$ = 6.8 Hz, $J_{2'-3'}$ = 5.3 Hz, 1, $H^{2'}$), 5.71 (d of d, $J_{3'-2'}$ = 5.3 Hz, $J_{3'-4'}$ = 1.7 Hz, 1, $H^{3'}$), 6.16 (d, $J_{1'-2'}$ = 6.8 Hz, 1, $H^{1'}$), 7.11 (d, *J* *ortho*-*meta* = 9.2 Hz, 2, aromatic protons), 7.40 (br s, 2, 6-NH_2), 8.05 (d, *J* *ortho*-*meta* = 9.2 Hz, 2, aromatic protons), 8.21, 8.48 (s,s; 1,1; H^2, H^8); ms (200°) m/e 415 (1, M), 385 (6, c), 384 (4, M-OCH_3), 281 (5, s), 280 (3.5 $M-CO\phi OCH_3$), 270 (0.5), 264 (0.6, $M-OCO\phi OCH_3$), 249 (1.5, $M-(OCH_3 + CO\phi OCH_3)$), 234 (3.5, x), 233 (2, $M-(OCH_3 + OCO\phi OCH_3)$), 232 (0.5, $M-(OCH_3 + HOCO\phi OCH_3)$), 202 (3.5), 192 (6.5, d), 177 (1,v), 176 (1), 174 (1), 173 (1.5, $y-OCH_3$), 164 (7.5,h), 162 (0.7), 152 (6, $HOCO\phi OCH_3$), 148 (5, f), 146 (1, $s-CO\phi OCH_3$), 145 (0.8, $w-CO\phi OCH_3$), 136 (31, B + 2H), 135 (100, B + H).

Anal. Calcd for $C_{19}H_{21}N_5O_6$: C, 54.93; H, 5.10; N, 16.86. Found: C, 55.04; H, 5.12; N, 16.48.

Product (54) of treatment of 9-(β-D-xylofuranosyl)adenine (58) with $SOCl_2$ in HMPA

The procedure described by Hogenkamp for preparation of 5'-deoxy-5'-chloro-ara-adenosine [9-(5-chloro-5-deoxy-β-D-arabinofuranosyl)adenine] [133] was followed.

To a stirred solution of 0.2 ml (0.0028 mole) of $SOCl_2$ in 2 ml of hexamethylphosphoramide (HMPA) was added 0.2 g (0.00075 mole) of 58. The solution was stirred for 12 h at 24° and 4 ml of H_2O was added. The resulting solution was applied to a column of Amberlite IR-120 (H^+) (1.5 x 10 cm). The column was washed with 300 ml of H_2O and the nucleosidic material was eluted with 0.1 N NH_3 (aqueous). The ammoniacal solution was concentrated to ~40 ml and extracted with $CHCl_3$ (3 x 50 ml) to remove residual HMPA. The aqueous layer was evaporated to dryness and 98% EtOH was added and coevaporated (2 x 20 ml) to remove residual H_2O. The residue was dissolved in a small amount of H_2O and EtOH and applied to two silica gel plates. The plates were developed in SSE solvent. The upper band (lower band was starting material) was eluted and evaporated to dryness to give 0.03 g (14%) of a product (54).

This product had mass spectrum (Calcd for $C_{10}H_{12}N_5O_3{}^{35}Cl$: 285.0629; Found 285.0639) m/e 285 (10.5, M), 250 (64, M-Cl), 249 (2, M-HCl), 236 (2, M-CH_2Cl), 232 (2, M-(Cl + H_2O)), 194 (b, i), 178 (32, d), 164 (70, h), 148 (9, f), 136 (91, B + 2H), 135 (100, B + H); nmr (DMSO-$\underline{\underline{d}}_6$ - D_2O) δ 3.77 (d of d, $\underline{J}_{5'-5''}$ = 11.0 Hz, $\underline{J}_{5'-4'}$ = 7.1 Hz, 1, $H^{5'}$), 3.93 (d of d, $\underline{J}_{5''-5'}$ = 11.0 Hz, $\underline{J}_{5''-4'}$ = 4.9 Hz, 1, $H^{5''}$), 4.09 (d of d, $\underline{J}_{3'-2'}$ = 1.6 Hz, $\underline{J}_{3'-4'}$ = 3.8 Hz, 1, $H^{3'}$), 4.33 (d of d of d, $\underline{J}_{4'-3'}$ = 3.8 Hz, $\underline{J}_{4'-5''}$ = 4.9 Hz, $\underline{J}_{4'-5'}$ = 7.1 Hz, 1, $H^{4'}$), 4.39 (t, $\underline{J}_{2'-1'}$ = $\underline{J}_{2'-3'}$ = 1.6 Hz, 1, $H^{2'}$), 5.91 (d, $\underline{J}_{1'-2'}$ = 1.6 Hz, 1, $H^{1'}$), 8.15, 8.26 (s,s;1,1; H^2, H^8):

Comparison of product (54) with starting material (58)

by ^1H nmr spectroscopy

(DMSO-$\underline{\underline{d}}_6$-$D_2O$) proton	54	Xylosyladenine	Δδ in Hz
5'	3.77	3.59*	18
5"	3.93	3.79*	14
4'	4.33	4.16	17
3'	4.09	4.02	7
2'	4.39	4.32	7

1'	5.91	5.84	7
2	8.15	8.15	0
8	8.26	8.26	0

*somewhat obscured by HOD peak, shifts obtained by comparison of (DMSO-d_6) nmr with (DMSO-d_6,D_2O) nmr.

Also when the nmr (DMSO-d_6-D_2O) sample of 54 was heated in boiling H_2O, new peaks appeared at δ 6.30 (s, 1, $H^{1'}$), 8.47, 8.70 (s,s; 1,1; H^2, H^8) indicating cyclonucleoside formation.

Electrophoresis on Whatman #1 paper in 0.05 M sodium borate (pH = 9) with 54 gave:

Cpds.	Distance Migrated Toward Anode in Mm
xylosyladenine	161
2'-deoxyadenosine	48
54	44
araadenosine	45
adenosine	140

1.5 kV   40 - 80 mA   90 min

Paper chromatography in the borate system gave:

Cpd.	Rf
xyloadenosine	.44
2'-deoxyadenosine	.72
54	.76

arabinosyladenine	.59
adenosine	.33

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APPENDIX

KINETICS DATA

All the following reactions were carried out at 81.4°C in 1.0 N HCl [1,4-dioxane : 2.5 N HCl (aqueous) (3:2)].

Hydrolysis of 3'-O-Mesyl (35) at Initial Concentration of 2.49×10^{-2} M Using Method A

Time Min	A max of Adenine A	A max of 35 B	Conc. Aden. $\times 10^5$ $C = A/13{,}100$	Conc. 35 $\times 10^5$ $D = B/15{,}500$	% 35 remaining $(\frac{D}{C+D}) \times 100$	$\ln(\frac{D+C}{D})$	$k' \times 10^5, \mathrm{sec}^{-1}$
0	0	1.224	0	7.90	100	0	-
15	0.079	1.240	0.603	8.00	93.0	0.0727	8.08
30	0.179	1.228	1.37	7.92	85.3	0.160	8.89
60	0.288	1.152	2.20	7.43	77.2	0.259	7.19
90	0.318	0.963	2.43	6.21	71.9	0.330	6.11
120	0.490	0.812	3.74	5.24	58.4	0.539	7.49
180	0.640	0.666	4.89	4.30	46.8	0.760	7.04
300	0.894	0.320	6.82	2.06	23.2	1.46	8.11
450	1.078	0.238	8.23	1.54	15.8	1.72	6.84

$$k' = 7.47\ (\pm 0.41) \times 10^{-5}\ \mathrm{sec}^{-1}$$

Hydrolysis of 3'-O-Mesyl (35) at Initial Concentration of 2.52×10^{-2} M Using Method B

Time min.	HPLC integr. of Adenine pK A	HPLC integr. of 35 B	Correction* of B for ε C	% 35 remaining $(\frac{C}{A+C}) \times 100$	$\ln\frac{A+C}{C}$	$k' \times 10^5, sec^{-1}$
0	0	38.75	34.9	100	0	-
60	5.32	27.86	25.1	82.5	0.192	5.33
105	16.87	32.84	29.6	63.7	0.452	7.17
120	12.03	23.86	21.5	64.2	0.443	6.15
150	16.33	24.23	21.8	57.2	0.558	6.20
180	22.72	23.33	21.0	48.1	0.733	6.79
210	29.33	22.93	20.6	41.3	0.885	7.02
240	42.43	26.76	24.1	36.2	1.01	7.01
270	28.26	15.72	14.1	33.3	1.10	6.79
300	32.23	13.88	12.5	28.0	1.27	7.06
360	34.84	11.53	10.4	23.0	1.47	6.81
420	26.49	4.89	4.40	14.2	1.95	7.74

$$k' = 6.73\ (\pm 0.46) \times 10^{-5}\ sec^{-1}$$

* multiply B by ratio of ε at 254 nm (i.e. B × $\frac{10,800}{12,000}$)

Hydrolysis of 2'-O-Mesyl (34) at Initial Concentration of 2.46×10^{-2} M

Using Method A

Time Min	% of 34 Remaining	$k' \times 10^5$, sec^{-1}
0	100	-
60	86.8	3.97
120	77.5	3.58
180	72.4	2.99
240	65.1	2.99
300	59.7	2.87
360	55.2	2.75
420	48.9	2.77
480	45.1	2.77
660	34.8	2.66
1380	12.3	2.53

$$k' = 2.99\ (\underline{+}0.32) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Mesyl (34) at Initial Concentration of 2.49×10^{-2} M

Using Method B

Time Min	% of 34 remaining	k' x 10^5, sec^{-1}
0	100	-
60	90.1	2.88
118	83.3	2.57
190	73.0	2.77
241	67.1	2.76
300	61.3	2.71
1357	13.1	2.49
1411	11.5	2.56

$k' = 2.68\ (\pm 0.12) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 2'-O-Methyl (32) at Initial Concentration of 2.47×10^{-2} M

Using Method B

Time min.	% of 32 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
1	83.4	299
2	73.3	261
3	61.1	274
4	53.5	260
5	47.3	252
7	35.1	249
9	25.5	253
11.5	18.2	247
14	14.3	231

$k' = 258\ (\pm 13) \times 10^{-5}\ \text{sec}^{-1}$

Hydrolysis of 2'-O-Methyl (32) at Initial Concentration of 2.49×10^{-2} M

Using Method B

Time Min	% of 32 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
1	86.3	246
2	74.1	249
3	63.6	251
4	54.4	253
6	46.6	212
8	31.1	243
10	22.2	251
12	16.6	249

$$k' = 244\ (\pm 9) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Methyl (17) at Initial Concentration of 2.49×10^{-2} M

Using Method B

Time min	% of 17 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
.5	88.2	422
1.5	64.8	484
2.5	50.6	456
3.5	38.9	450
4.5	30.8	437
5.5	23.1	445
6.5	17.8	443
9.0	12.0	393

$$k' = 441\ (\pm 18) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Methyl (17) at Initial Concentration of 2.50 x 10^{-2} M

Using Method B

Time min	% of 17 remaining	k' x 10^5, sec^{-1}
0	100	-
.5	86.4	484
1.5	66.8	450
2.5	47.1	501
3.5	38.7	450
4.5	31.1	432
5.5	22.8	448
6.5	17.6	446
7.5	13.3	448
8.5	11.4	427

$k' = 454\ (\pm\ 17) \times 10^{-5}\ \text{sec}^{-1}$

Hydrolysis of 2'-O-Benzyl (47) at Initial Concentration of 2.48×10^{-2} M

Using Method B

Time min	% of 47 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
1.5	84	189
4	68	160
9	40.6	165
14	26.6	157
19	17.8	152
24	9.92	160

$$k' = 164\ (\pm\ 9) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Benzyl (47) at Initial Concentration of 2.50×10^{-2} M

Using Method B

Time min	% of 47 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
2	83.0	154
4	70.7	144
6	55.7	162
8	48.6	150
10	37.7	162
12	29.7	169
14	23.2	174
17	17.83	169
21	13.33	160
23	11.36	158

$k' = 160\ (\pm 8) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 3'-O-Benzyl (46) at Initial Concentration of 2.45×10^{-2} M

Using Method B

Time min	% of 46 remaining	$k' \times 10^5$, sec^{-1}
0	100	
1	82.3	319
3	59.0	292
5	42.0	289
7	33.3	262
9	24.1	264
11.5	14.2	283
14	10.2	272

$$k' = 283\ (\pm 15) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Benzyl (46) at Initial Concentration of 2.45×10^{-2} M

Using Method B

Time min	% of 46 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
1	84.5	280
2	72.1	274
3	59.7	288
4	55.6	245
5	47.5	249
7	28.0	303
9	21.7	283
11	15.1	286
13	10.8	286

$$k' = 277\ (\pm 11) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Tosyl (38) at Initial Concentration of 2.48×10^{-2} M

Using Method A

Time min	% of 38 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
120	92.9	1.02
210	86.9	1.13
420	74.5	1.17
430	73.6	1.19
1140	47.7	1.08
1560	36.1	1.09
1830	32.2	1.03
2610	17.4	1.12
2700	17.3	1.08

$$k' = 1.10\ (\pm 0.05) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Tosyl (38) at Initial Concentration 2.50×10^{-2} M

Using Method A

Time min	% of 38 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
360	81.4	0.96
375	80.0	0.99
1560	38.9	1.01
1590	36.2	1.07
2760	15.9	1.11
2775	16.2	1.09
2805	16.4	1.08
2835	16.3	1.07
2840	16.1	1.07

$$k' = 1.05\ (\pm 0.04) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Tosyl (39) at Initial Concentration of 2.49×10^{-2} M

Using Method A

Time min	% of 39 remaining	k' × 10^{5}, sec^{-1}
0	100	-
60	85.3	4.48
120	74.3	4.09
180	65.0	3.99
240	59.1	3.65
310	52.2	3.49
330	48.2	3.69
675	18.8	4.13
695	17.6	4.16
725	20.2	3.68

$$k' = 3.93\ (\pm 0.25) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Tosyl (39) at Initial Concentration of 2.52×10^{-5} M

Using Method A

Time min	% of 39 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
60	86.9	3.90
120	77.4	3.55
240	57.4	3.84
360	47.8	3.42
480	32.7	3.88
1440	2.5	4.26

$$k' = 3.81\ (\pm 0.22) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Besyl (36) at Initial Concentration of 2.51×10^{-2} M

Using Method B

Time min	% of 36 remaining	k' x 10^5, sec^{-1}
0	100	-
170	83.3	1.78
240	76.5	1.86
300	72.7	1.77
360	69.7	1.67
420	62.2	1.88
480	62.2	1.65
600	54.3	1.70
725	47.5	1.71
1530	24.7	1.53
1565	23.4	1.55
1635	20.0	1.64
1675	23.0	1.46

$$k' = 1.68\ (\underline{+}0.10) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Besyl (36) at Initial Concentration of 2.49×10^{-2} M

Using Method B

Time min	% of 36 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
60	93.3	1.92
165	83.3	1.83
195	81.5	1.75
255	76.7	1.73
310	72.6	1.72
580	50.8	1.95
600	53.2	1.75
820	41.1	1.81
840	41.8	1.73
1530	22.2	1.64
1560	21.4	1.65

$k' = 1.77 (\pm 0.08) \times 10^{-5}$ sec^{-1}

Hydrolysis of 3'-O-Besyl (37) at Initial Concentration of 2.54×10^{-2} M

Using Method B

Time min	% of 37 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
30	90.3	5.62
60	83.1	5.18
90	74.6	5.42
125	66.8	5.37
155	61.0	5.32
180	56.0	5.37
210	48.9	5.67
250	44.1	5.45
300	37.8	5.40
320	34.6	5.53
540	19.4	5.07
570	15.7	5.41
810	8.96	4.95

$$k' = 5.37\ (\underline{+}0.14) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Besyl (37) at Initial Concentration of 2.48×10^{-2} M

Using Method B

Time min	% of 37 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
60	82.2	5.44
90	74.6	5.42
120	66.3	5.69
150	62.2	5.27
180	56.9	5.22
210	49.5	5.57
240	45.5	5.47
270	39.4	5.74
300	41.9	4.84
420	26.6	5.26
540	19.3	5.08

$k' = 5.36\ (\pm 0.21) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 2'-O-Nisyl (40) at Initial Concentration of 2.53×10^{-2} M

Using Method A

Time min	% of 40 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
120	87.3	1.86
180	84.2	1.60
405	80.1	0.92
1170	50.9	0.96
1200	53.8	0.86
1230	51.3	0.90
1380	46.6	.92
1545	42.6	.92
1800	36.2	.94
1815	35.8	.94
2730	22.5	.91
2970	22.2	.85

$k' = 1.05\ (\pm 0.21) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 2'-O-Nisyl (40) at Initial Concentration of 2.47×10^{-2} M

Using Method A

Time min	% of 40 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
570	76.2	0.79
585	68.8	1.06
1410	51.8	0.78
1440	51.7	0.77
1470	44.8	0.91
1740	43.0	0.81
1755	37.1	0.94
2955	22.7	0.84
2965	22.4	0.84

$k' = .86\ (\pm\ 0.07) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 3'-O-Nisyl (41) at Initial Concentration of 2.47×10^{-2} M

Using Method A

Time min	% of 41 remaining	$k' \times 10^5, sec^{-1}$
0	100	-
60	89.1	3.13
120	76.9	3.65
180	71.6	3.09
245	62.7	3.18
300	55.9	3.22
360	53.2	2.91
420	44.7	3.20
460	40.5	3.27
700	29.1	2.94
720	26.6	3.07

$k' = 3.17\ (\pm 0.14) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 3'-O-Nisyl (41) at Initial Concentration of 2.46×10^{-2} M

Using Method A

Time min	% of 41 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
130	82.4	2.42
210	68.9	2.96
315	51.9	3.46
375	50.1	3.07
390	50.4	2.93
405	46.6	3.15
465	41.4	3.16
660	28.0	3.22
675	27.1	3.23
690	25.4	3.32

$$k' = 3.09\ (\pm 0.19) \times 10^{-5}\ \text{sec}^{-1}$$

Hydrolysis of 2'-O-Aminosyl (42) at Initial Concentration of 2.49×10^{-2} M

Using Method A

Time min	% of 42 remaining	$k' \times 10^5$, sec^{-1}
0	100	-
140	89.7	1.29
300	77.1	1.46
350	73.8	1.45
370	71.3	1.51
395	70.7	1.47
600	58.9	1.46
620	61.7	1.29
1420	24.2	1.67
1450	21.8	1.75
1500	21.9	1.69
1570	17.7	1.84
1700	18.1	1.68
1779	15.7	1.74
1785	15.7	1.73
2985	4.54	1.73

$$k' = 1.58\ (\pm 0.15) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 2'-O-Aminosyl (42) at Initial Concentration 2.51 x 10^{-2} M

Using Method A

Time mine	% of 38 remaining	k' x 10^5, sec^{-1}
0	100	-
180	84.7	1.54
345	77.4	1.23
1140	31.5	1.69
1380	27.0	1.58
1440	25.8	1.57
1500	24.1	1.58
1770	20.6	1.49
1785	19.0	1.55

$$k' = 1.53\ (\pm 0.08) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Aminosyl (43) at Initial Concentration of 2.48×10^{-2} M

Using Method A

Time min	% of 43 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
100	76.0	4.57
120	71.2	4.70
150	66.7	4.50
180	60.2	4.65
240	50.5	4.75
285	45.8	4.57
300	44.0	4.54
360	36.9	4.60
575	21.7	4.43
580	19.1	4.74

$$k' = 4.61\ (\pm 0.09) \times 10^{-5}\ sec^{-1}$$

Hydrolysis of 3'-O-Aminosyl (43) at Initial Concentration of 2.48×10^{-2} M

Time min	Using Method A % of 43 remaining	$k' \times 10^{5}$, sec^{-1}
0	100	-
120	67.6	5.44
150	67.2	4.43
180	58.0	5.05
240	49.0	4.94
320	42.5	4.46
330	38.6	4.80
350	37.2	4.70
390	31.8	4.89
435	29.3	4.70
450	28.4	4.66
500	25.2	4.59
530	23.8	4.52
540	22.1	4.66
545	23.0	4.50

$k' = 4.74\ (\pm 0.21) \times 10^{-5}\ sec^{-1}$

Hydrolysis of 2' Triflate (44) at Initial Concentration of 2.49×10^{-2} M

Using Method A

Time min	% (44)	% biproduct	% Adenine
0	100	0	0
2.5	95.4	1.9	2.8
5	88.3	4.2	7.5
10	78.2	8.2	13.6
15	68.5	11.3	20.1
20	59.9	12.3	27.8
25	53.6	12.9	33.6
30	48.1	12.5	39.4
35	42.1	14.7	43.2
40	37.9	13.9	48.3
50	29.1	13.1	57.8
60	29.4	11.7	64.9
80	15.3	9.1	75.6
100	12.1	7.7	80.2
120	9.8	8.4	81.8

Hydrolysis of 3' Triflate (45) at Initial Concentration of 2.48×10^{-2} M

Using Method A

Time min	% (45)	% biproduct	% Adenine
0	100	0	0
1	76.9	10.0	13.1
2	63.5	14.8	21.7
3	49.0	20.8	30.3
4	40.4	23.5	36.1
6	27.9	26.8	45.3
8	19.6	29.3	51.1
10	16.2	31.1	52.7
12.5	10.0	31.0	59.0
15	10.2	31.2	58.6

Mobility of Sugar Substituted Adenosine Derivatives on Paper Chromatography

Abbreviated Name	Compound Number	Rfs: IPA/H_2O/NH_3	Rfs: EtOH/H_2O
2'-*O*-Methyl	32	0.64	0.62
3'-*O*-Methyl	17	0.61	0.55
2'-*O*-Benzyl	47		0.68
3'-*O*-Benzyl	46		0.68
2'-*O*-Mesyl	34	0.66	
3'-*O*-Mesyl	35	0.68	
2'-*O*-Tosyl	38	0.82	0.72
3'-*O*-Tosyl	39	0.83	
2'-*O*-Nisyl	40		0.65
3'-*O*-Nisyl	41	0.79	0.71
2'-*O*-Triflate	44		0.73
3'-*O*-Triflate	45		0.81
2'-*O*-Aminosyl	42		0.73
3'-*O*-Aminosyl	43		0.74
3'-*O*-Triflate Biproduct	53		0.70
2'-*O*-Triflate Biproduct	52		0.65
Adenine	1		0.55
Sulfanilic acid			0.62